1982

# cancer treatment reports

February 1982
Volume 66
Number 2
Pages 217-415

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

# cancer treatment reports

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Third Annual Meeting of the American College of Clinical Pharmacy
Fourteenth Meeting of the International Society for Pediatric Oncology (SIOP)
Third EORTC Breast Cancer Working Conference
Second International Drug Conference
Position Vacancy: Associate Director, Cancer Therapy Evaluation Program
Cancer Control and the Primary Physician

# Statistical Guidelines for Medical Research Reports[1]

**Robert W. Makuch**[2,*]

A number of prominent statisticians have expressed concern regarding the lack of attention toward the statistical aspects of medical research publications (1,2). Since the areas susceptible to statistical deficiencies range from the initial design of the experiment to the final analysis and description of results, proper reporting of the statistical considerations involved in the planning, execution, and analysis of a research study serves many useful purposes. Most importantly, a reasonable level of documentation is required so that readers can make sound and informed judgments regarding the quality of work performed and the extent to which research objectives have been met. In addition, disclosure of statistical aspects of a study provides information that other investigators can use to design additional experiments.

From another viewpoint, adequate descriptions of the analytic and design characteristics of a study can play a key role in subsequent literature reviews. If controversies about study outcomes arise among different investigators pursuing similar lines of research, knowledge of the statistical aspects may be crucial in determining whether the experiments differ in ways sufficient to explain their conflicting results. Data description is useful to evaluate comparability of patient characteristics between experiments testing a similar hypothesis. For these reasons, recommendations are provided below for important statistical features that should be included in reports submitted for publication in *Cancer Treatment Reports*.

## Study Design

If the study design includes randomization, the method of randomization should be discussed. In particular, it is useful to include the technique used to generate the randomization list (eg, stratified randomization or dynamic allocation) and to explain how the rules for patient assignment were provided to the investigator (eg, centrally located telephone randomization or sealed envelope opened by the investigator). It is also important to specify whether the study was single-blind, double-blind, or not blind. If a sequential, group sequential, or early termination rule was employed, a reference for the particular scheme used should be cited.

If historic controls were used for comparative purposes, this should be stated and, if available, a relevant reference regarding the source of historic control information should be provided. Otherwise, the selection factors used to derive the historic control group should be given. Other important design features, such as the dose-escalation technique used for phase I studies, should be reported or an appropriate reference should be provided. A definition of each type of toxicity considered in the analysis or a reference that adequately defines the criteria should be stated. Definitions of responses also should be indicated unless a reference can be provided in which complete definitions are given.

## Reporting *P* Values

The actual $P$ value associated with a statistical test should be provided; a statement such as "$P < 0.05$" is not sufficient. In addition, the statistical test used should be identified for each general type of analysis performed, and a reference should be provided for each test unless there is no ambiguity concerning the exact form of the test (eg, t test, F test, or chi-square test). Significance levels should be specified as one-sided or two-sided.

## Time-to-Failure Curves

The method used to derive time-to-failure curves should be specified. Either the life-table method (3) or the Kaplan-Meier product-limit procedure (4) is sufficiently well-known that no description or acknowledgement in the reference section is required. Any other methods that are used should be referenced or described in full. A clear, complete definition of the endpoints on which analyses are based should be included; for example, if death is considered as the failure type, it is necessary to specify whether observations of patients who die of causes other than those related to the malignancy are treated

[1]Received Apr 30, 1981; revised June 29, 1981; accepted June 29, 1981.

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as censored or uncensored data in the analysis. Similarly, if disease-free survival is used as an endpoint, it should be stated whether data on patients who die disease-free are censored or uncensored. For the purpose of clarity, the phrase "disease-free interval" is recommended if data on patients who die disease-free are considered censored.

## Methods of Analysis

If statistical tests are used to analyze the major endpoints of a study (eg, response rates in phase II studies) in which the power of the tests is relatively simple to calculate (eg, t test, F test, or chi-square test), then the power of the test should be reported. The power gives some idea of the chance of finding differences of a size that one believes are of practical importance. Mosteller et al (1) suggest that one possible mechanism is to report the effect that would be detected 50% of the time according to the design and statistical method used. In large studies, 80% or 90% power calculations could be given instead. Another possibility would be to calculate 90% confidence intervals for the important quantifiable endpoints.

The comparison of time-to-failure curves at one point in time, ignoring their structure elsewhere, is usually inefficient. Statistical methods that take into account and compare the entire curve are preferred. Factors which have a strong influence on the outcome under study and could potentially affect treatment comparisons should be considered as covariates or stratification variables in the analysis. Reports should include the steps taken to adjust for important prognostic factors. In all statistical analyses of censored or uncensored data, the data should be examined to ensure that the underlying assumptions associated with the proper use of the statistical test are not violated.

## Data Display

In full-length papers, investigators who have small data sets ($\leq$ 30 patients) are encouraged to submit all pertinent data in tabular form. In such circumstances the best summarization of the data may be the data themselves. When the number of individual data points becomes too large for complete tabulation, summarization of the data, using scatter plots and other graphic displays together with the usual summary statistics (eg, mean or standard deviation) can offer valuable insights into the experimental results.

For the display of time-to-failure curves, the entire curve should be presented unless the analysis includes a large number of curves, as in the case of prognostic factor studies. In this situation, median failure times are a possible alternative measure to summarize the data, although one must recognize that they are very unreliable unless the failure rate around the median failure time is still high (5). The proportion of failures selected from the curve at a specified time point may also be used, although a specified time point should not be selected merely because the curves differ the most at that time. In either case, it is recommended that confidence limits be included since simple methods to calculate such limits now exist (eg, ref 6).

## Bias

For comparative trials, investigators are urged to analyze all patients initially entered in the study and to analyze them in the treatment group to which they were originally assigned. This should eliminate the potential for serious biases to be introduced into treatment comparisons by withdrawal of patients after treatment assignment or by switching patients to treatment groups other than the group initially assigned.

In some instances, patients are entered in the study and found nonevaluable in a subsequent review of patient records. If this subgroup of patients is excluded from the analysis presented in the report, the number of nonevaluable patients and reasons for their exclusion should be presented. *P* values based on data excluding protocol deviants or other categories of withdrawn patients should be carefully interpreted (5). Thus, if analyses based on selected subgroups of patients are included in the report, they should receive less emphasis than results derived from all patients. Likewise, for phase II studies, all patients who began therapy should be discussed to some extent. To totally disregard patients who, for example, failed very early during their treatment schedule can lead to overly optimistic response rates.

## Generalizability

Investigators are urged to describe in some detail (perhaps in a table) the pretreatment patient characteristics of the studied population. Others may then be able to better determine the particular population of cancer patients to whom the particular treatment outcomes apply.

## Conclusion

Requirements for clear and complete documentation of statistical aspects of a research project not only should lend greater credibility to final reports

but also should spur investigators to weigh carefully the statistical aspects of planning and analyzing the study results. An excellent and more extensive review of design and analytic techniques is given by Peto et al (5).

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# Combination Chemotherapy for Small Cell Carcinoma of the Lung: Continuous Versus Alternating Non-Cross-Resistant Combinations[1,2]

Joseph Aisner,* Margaret Whitacre, David A. Van Echo, and Peter H. Wiernik[3,4]

## SUMMARY

**After stratification for extent of small cell lung cancer, 109 patients were randomized to receive cycles of chemotherapy with cyclophosphamide, doxorubicin, and VP-16-213 [CAVP16 (regimen I)] or to receive CAVP16 to maximum response (minimum of three courses) and then chemotherapy with CCNU, methotrexate, vincristine, and procarbazine (COMP) alternating with CAVP16 (regimen II). A group of patients who achieved complete remission were randomized to receive whole-brain irradiation or to have observation only. Of the 44 patients with limited disease, 28 (64%) achieved a complete remission and 11 (26%) achieved a partial remission. Of the 65 patients with extensive disease, 26 (40%) achieved a complete remission and 28 (46%) achieved a partial remission. There were no significant differences between the regimens in response or survival. The projected median survival times are 14 and 10 months for limited and extensive disease, respectively. Nearly 30% of patients with limited disease will be 2-year, disease-free survivors. Twenty-nine patients were randomized to receive cranial irradiation or observation only; none of the 15 irradiated patients developed cerebral metastases, but five of 14 randomized to observation relapsed in the brain ($P = 0.02$). One patient died with necropsy evidence of only intracranial disease. The principal hematologic toxic effect was leukopenia. There were 31 febrile episodes (21 infectious) during neutropenia and four toxic deaths. Nonhematologic toxicity was mild. Cranial irradiation in patients who achieve complete remission delays or reduces the incidence of CNS metastases. Although alternating chemotherapy is not beneficial, combination chemotherapy with CAVP16 alone is a highly effective treatment modality for small cell carcinoma of the lung, regardless of stage.**

[Cancer Treat Rep 66:221–230, 1982]

Small cell carcinoma of the lung is a distinct clinical and pathologic entity with unique biologic and clinical behavior (1–4). The application of aggressive combination chemotherapy with or without adjuvant radiotherapy has resulted in improved survival for patients with limited and extensive disease, including some who are long-term, disease-free survivors (5–17). Most of these studies have shown that patients who achieve complete remission (CR) survive significantly longer than patients who do not. Our previous study utilizing the three-drug combination of cyclophosphamide, doxorubicin, and VP-16-213 (CAVP16) resulted in a high (> 50% overall) CR rate, an overall median survival > 12 months, and a fraction of long-term, disease-free survivors (6). Results with this chemotherapy combination compare favorably to results of any study in which,

[1]Received Aug 7, 1981; accepted Aug 11, 1981.

[2]Presented in part at the 16th Annual Meeting of the Society for Clinical Oncology, May 27, 1980, San Diego, CA, and at the Second World Conference on Lung Cancer, June 10, 1980, Copenhagen, Denmark.

[3]Baltimore Cancer Research Center, MD.

[4]We acknowledge the assistance of Ms. Nancy Brandt with data analysis and thank Ms. Carol L. McNamara for preparation of the manuscript.

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in addition to chemotherapy, irradiation was given to the primary tumor (3,5,7,17). Some patients who failed to achieve CR may have done so because they had tumors refractory to the combination of drugs used. In an attempt to improve the CR rate, the current study was therefore undertaken to test a second non-cross-resistant combination of drugs as part of an alternating treatment regimen given after maximal response with the first combination (CAVP16) had been achieved. Selection of the second combination of CCNU, methotrexate, vincristine, and procarbazine (COMP) was based on the facts that the single agents had antitumor activity, that the combination was similar to multiple regimens used in other studies (3,5), and that it had shown about a 30% objective response rate in our patients who were refractory to, or relapsing from, initial primary treatment.

One of the frequent complications of this disease is the occurrence of intracranial metastases (18–24). Most studies have suggested that the application of "prophylactic" cranial irradiation to treat micrometastases within the brain is effective in reducing or delaying the clinical expression of such metastases (5,16–22). There are, however, conflicting data about the effect of this irradiation (25) and there is no evidence that such cranial irradiation improves survival. Therefore, an additional goal of this study was to utilize "prophylactic" cranial irradiation given after the patient had achieved CR to test if this might be the optimal time to administer such radiation therapy.

## MATERIALS AND METHODS

All patients admitted to the Baltimore Cancer Research Center from July 1977 to August 1980 with small cell carcinoma of the lung were extensively staged using history and physical examination; bronchoscopy with brushings, washings, and biopsies; whole-lung tomography, computerized tomographic (CT) scan, or both; isotopic liver and bone scans (with roentgenograms of suspicious bone lesions), bilateral iliac crest bone marrow aspirations, and biopsies; CT brain scan, cbc, platelet count, and differential; serum chemistries including electrolytes, liver function studies (SGOT, SGPT, LDH, and alkaline phosphatase), and uric acid; and assays for biomarkers such as ACTH and ADH where clinically indicated. Following staging, patients were stratified according to extent of disease. Limited disease was defined as disease confined to one hemithorax with or without bilateral mediastinal lymph node involvement and with or without ipsilateral supraclavicular and cervical node involvement (single irradiation port). Extensive disease was defined as any involvement beyond the confines of the definition for limited disease including pleural effusion. There were no age restrictions and all patients were considered eligible for study if they gave informed consent and did not have any evidence of other primary malignancies.

After evaluation and stratification, patients were randomized to receive one of two treatment regimens. CAVP16 (regimen I) consisted of cyclophosphamide (1000 mg/m$^2$ iv on Day 1), doxorubicin (45 mg/m$^2$ iv on Day 1), and VP-16-213 (50 mg/m$^2$ iv on Days 1–5). Cycles were repeated every 21 days. The other treatment (regimen II) consisted of CAVP16 as above, given to maximum response (minimum of three courses; *see* below) followed by COMP consisting of CCNU (75 mg/m$^2$ orally on Day 1), vincristine (1 mg/m$^2$ iv on Days 1 and 21), methotrexate (40 mg/m$^2$ iv on Days 1 and 21), and procarbazine (50 mg/m$^2$ orally on Days 1–5 and 21–25). The 56-day cycles of COMP were alternated with 21-day cycles of CAVP16. Both regimens were given for 2 years or until there was disease progression. All patients randomized to the alternating regimen were given a minimum of three courses of CAVP16 unless there was unequivocal evidence of disease progression. Patients with disease progression were given the alternate regimen. After three courses, patients were re-evaluated to determine level of response. Those patients who had achieved a CR were given alternating chemotherapy. Those patients who had achieved a stable partial response (PR) as defined by no further changes in re-evaluation parameters (chest roentgenogram, chest CT scan, liver scan, bone marrow biopsies, etc) for at least two courses were also given the alternating chemotherapy. Patients who continued to show further tumor regression with CAVP16 continued to receive this regimen until there was no further regression for two successive courses; they then began to receive the alternating regimen. Patients who had disease progression while receiving regimen I were then given the alternate chemotherapy regimen. Patients who had disease progression while receiving regimen II were removed from study and given other therapy. The study was closed to patient entry in August 1980 and was analyzed in December 1980. The median followup time for all patients was 20 months. Response data were analyzed by the Fisher exact test, and survival was analyzed according to Wilcoxon analysis.

Patients were completely re-evaluated after the third course of chemotherapy for any change in clinical status, such as apparent CR or disease progression. This re-evaluation included history and physical examination and repeat of all previously positive or suspicious tests including whole-lung

TABLE 1.—Continuous (regimen I) vs alternating (regimen II) combination chemotherapy: patient characteristics*

| | Limited disease | | Extensive disease | |
|---|---|---|---|---|
| | Regimen I | Regimen II | Regimen I | Regimen II |
| No. of patients | 21 | 23 | 32 | 33 |
| Median age in yrs (range) | 63 (31–73) | 63 (50–76) | 59 (29–79) | 59 (48–69) |
| Males/females | 16/5 | 14/9 | 22/10 | 25/8 |
| Performance status† | | | | |
| 0 | 6 | 3 | 0 | 5 |
| 1 | 7 | 10 | 12 | 12 |
| 2 | 5 | 6 | 9 | 6 |
| 3 | 3 | 4 | 9 | 6 |
| 4 | 0 | 0 | 2 | 4 |

*Unless specified otherwise, values = No. of patients.
†CALGB scale. 0 = asymptomatic; 1 = fully ambulatory; 2 = ambulatory and asymptomatic but < 50% of waking time in bed; 3 = ambulatory with > 50% of time in bed; and 4 = 100% bedridden.

tomography or CT scan, bronchoscopy, isotopic liver and bone scans, CT brain scan, and bilateral iliac crest bone marrow aspirates and biopsies. No patient was judged as having achieved CR unless all of the repeat evaluations revealed no evidence of disease. Standard Cancer and Leukemia Group B (CALGB) criteria for response were used. CR was defined as the disappearance of all signs and symptoms of disease, including normalization of abnormal biomarkers lasting a minimum of 30 days. PR was defined as a minimum decrease of 50% in the cross-perpendicular dimensions of each lesion, with subjective improvement and no evidence of disease progression, lasting a minimum of 30 days. Minor response was defined as any tumor regression which did not fulfill the criteria of CR or PR. No response was defined as disease stabilization (no objective regression) or increased tumor growth during therapy.

All patients without prior cranial metastases who achieved CR were asked to participate in a randomized study of "prophylactic" cranial irradiation for the treatment of possible micrometastases in the brain. Consenting patients were randomized to receive 3000 rad of whole-brain irradiation given in ten fractions, while those who were randomized to have observation only were followed on a routine basis with CT scans. Patients who refused randomization to this treatment modality were observed similarly.

All patients were seen on at least a weekly basis to determine blood cell counts and potential toxic effects. Some patients participated in a randomized double-blind study of infection prevention utilizing trimethoprim/sulfamethoxazole or placebo. The preliminary results of that study have already been reported (26).

Radiotherapy to primary or distant sites was not administered during protocol treatment.

## RESULTS

### Patient Characteristics

Between July 1977 and August 1980, 109 patients were entered in the study; the individual patient characteristics are shown in table 1. The groups for regimens I and II are well-matched for performance status, numbers of patients, age, sex, and limited and extensive disease. There were no age restrictions, and patients up to age 79 were included in this study. In general, this was an ambulatory group of patients, none of whom had had any prior therapy. Sites of involvement were well-matched between the two treatment regimens (table 2). Most patients had identifiable lesions in the lung and regional nodes. The most common extrathoracic sites of metastases were liver, bone marrow, and bone. Of the patients with bone lesions, none had single isolated lesions detected only by bone scan.

### Response

Response to therapy according to treatment regimen is shown in table 3. There are no significant differences in CR or PR rates between the two treatments for either limited or extensive disease. Of the 44 patients with limited disease, 28 (64%)

TABLE 2.—Sites of disease involvement in 65 patients with extensive disease at presentation

| Site | No. of patients | |
|---|---|---|
| | Regimen I | Regimen II |
| Lung | 32 | 32 |
| Regional nodes | 32 | 26 |
| Distant nodes | 5 | 3 |
| Liver | 13 | 18 |
| Bone | 10 | 13 |
| Bone marrow | 15 | 16 |
| CNS | 7 | 5 |
| Other* | 4 | 2 |

*Pleura, pancreas, or soft tissue.

achieved CR and 11 (26%) achieved PR, for an overall response rate of 90%. The median duration of response was similar for both arms: 6½ months for patients with extensive disease (range, 1–18 months) and 10 months for patients with limited disease (range, 3–43+ months). Only three patients with limited disease failed to respond to therapy and 11 patients with limited disease remain in CR from 9½+ to 43+months. Of the 65 patients with extensive disease, 26 (40%) achieved CR and 28 (46%) achieved PR, for an overall response rate of 86%. Six patients with extensive disease failed to respond to induction therapy, and six patients remain in CR from 7+ to 24 months. The median number of courses to maximal response for patients receiving the alternating arm was three (range, one to seven), and the median number of courses to CR for all patients was between two and three (range, one to seven). None of the patients who achieved only PR developed a CR after switching to the alternating regimen. Six patients receiving regimen II (alternating) had progressive disease after switching to the alternating regimen: one patient had a short stabilization with CAVP16, but the other five patients continued to show progression and were removed from the study. Patients receiving regimen I (continuous), who progressed while being treated with CAVP16, were treated with the alternating regimen, which produced a 31% response rate of short duration (range, 1–6 months). Two of the six patients who did not respond to the "induction" arm had a short PR to the alternating regimen.

#### *Sites of Relapse—Progression*

In general, disease tended to progress (in partial responders) or relapse (in complete responders) in sites of previous disease. In most cases, however, patients relapsed in multiple sites. Among the 17 patients with limited disease who relapsed, eight did so in the chest, but five of the eight had simultaneous relapse in other sites such as liver, bone, or CNS. Three of the 17 patients relapsed in the chest only, but two of the three had systemic metastases occur within 6 weeks of the time of relapse. Among 20 patients with extensive disease in CR who relapsed, six relapsed in the chest but five of the six had other evidence of disease on evaluation and the other patient had evidence of systemic metastases within 6 weeks.

#### *Survival*

There were no significant differences ($P = 0.18$) in the survival curves between the two treatment regimens for patients with limited disease (fig 1). The projected median survival for patients receiving CAVP16 only is 16 months, and the projected median survival for the alternating regimen is 12 months; both curves plateau at approximately the same level. However, one patient receiving regimen II, who did not receive prophylactic cranial irradiation, developed a cerebral metastasis which progressed despite 6000 rad of irradiation. The patient died, and necropsy confirmed that disease was confined to the brain. Survival curves for the two treatment regimens for patients with extensive disease are also not significantly different ($P = 0.20$); the median survival times for patients receiving regimens I and II are 11 and 8 months, respectively.

TABLE 3.—Regimen I (continuous) vs regimen II (alternating) for small cell lung cancer: response to therapy

| | Limited disease | | | Extensive disease | | |
|---|---|---|---|---|---|---|
| | Regimen I | Regimen II | All | Regimen I | Regimen II | All |
| CR | 13 (62)* | 15 (65) | 28 (64) | 14 (44) | 12 (36) | 26 (40) |
| PR | 5 (25) | 6 (26) | 11 (26) | 12 (38) | 16 (50) | 28 (46) |
| Minor response | 0 | 2 | | 2 | 2 | |
| No response | 3 | 0 | | 3 | 3 | |
| Total | 21 | 23 | | 32 | 33 | |
| Still in study | 6 | 5 | | 3 | 4 | |

*Values = No. (%) of patients responding.

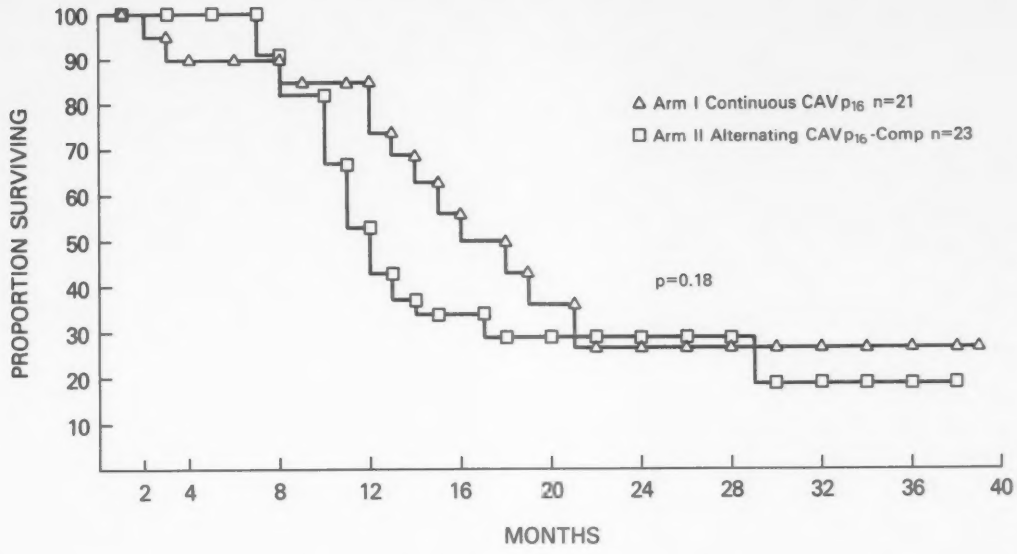


FIGURE 1.—Projected survival curves for patients with limited disease treated with either continuous CAVP16 (regimen I) or CAVP16 alternating with COMP (regimen II). Median survival times were 16 and 12 mos, respectively, but were not significantly different. At 30 mos, one patient receiving regimen II died of intracranial disease. It is conceivable that if this patient had received prophylactic cranial irradiation the patient would still be disease-free.

When the survival times of all patients with limited and extensive disease who received regimen I are compared with those of all patients who received regimen II, there is a trend ($P = 0.11$) in favor of the continuous chemotherapy (regimen I).

Patients who had achieved CR survived significantly longer than patients who did not. The projected median survival for patients with limited disease who had achieved CR is 18 months, compared to 11 months for patients who had not achieved CR (fig 2). The survival curve plateaus at 42% for patients who had achieved CR. The one patient who died with CNS disease only reduces the projected plateau at 30 months. So far, there have been no systemic relapses in any of the eight patients who have reached this plateau. A similar survival advantage is seen for patients with extensive disease who achieved CR. The projected median survival for patients with extensive disease who achieved CR is 14 months, compared to 7 months for those who did not ($P < 0.001$). So far, no patient with extensive disease has survived beyond 24 months.

The projected median survival for all patients with limited disease is 14 months and the plateau in the curve occurs at 29% (fig 3). As noted above, one patient died with only CNS disease at 30 months. The survival curve for all patients with limited disease is significantly better than for all patients with extensive disease ($P < 0.001$).

## Prophylactic Cranial Irradiation

Twelve patients with extensive disease presented with cranial metastases and they received 3000 rad of cranial irradiation in ten fractions as therapeutic irradiation. These patients were excluded from the prophylactic cranial irradiation. Prior to the interim analysis, 40 patients had achieved CR and were eligible for randomization to prophylactic cranial irradiation. Eleven patients refused randomization to cranial irradiation because of the possibility of permanent hair loss. Among the 29 patients who were randomized, 15 received irradiation and 14 were observed. At the time of the interim analysis, none of the 15 patients who received cranial irradiation had CNS metastases, while five of the 14 who were in the observation group developed intracranial metastases ($P < 0.02$). One of the patients who developed intracranial metastases died of this complication, without evidence of systemic relapse at necropsy. Among the 11 patients who refused cranial irradiation, four developed CNS relapse and if these 11 patients who did not receive cranial irra-

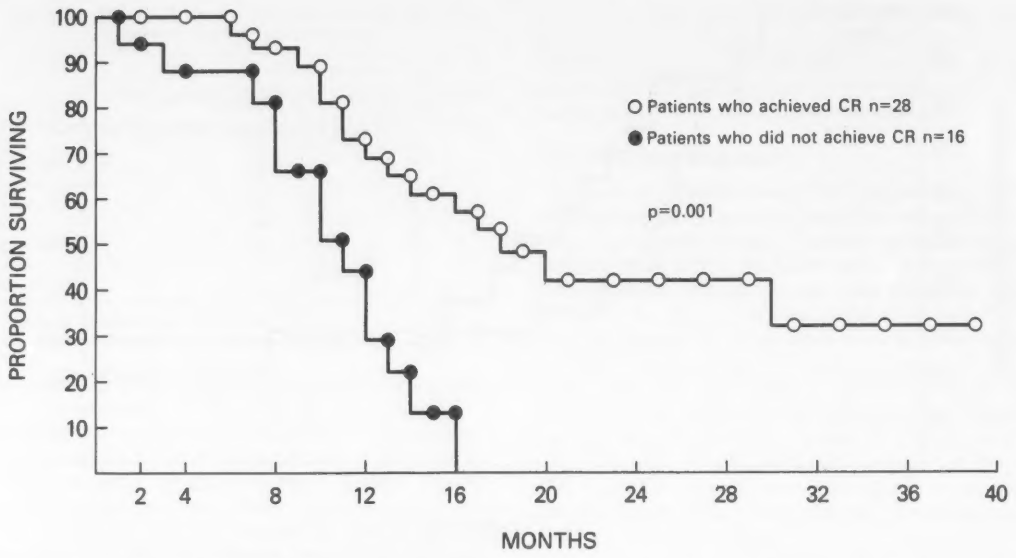


FIGURE 2.—Projected survival curves for patients with limited disease according to achievement of CR. Patients who achieved CR had significantly longer median survival (18 mos) compared to those who did not (11 mos) ($P = 0.001$). There have been no systemic relapses in any patient who finished 24 mos of therapy. At 30 mos, however, 1 patient died of brain metastases which proved to be the only evidence of disease at necropsy.

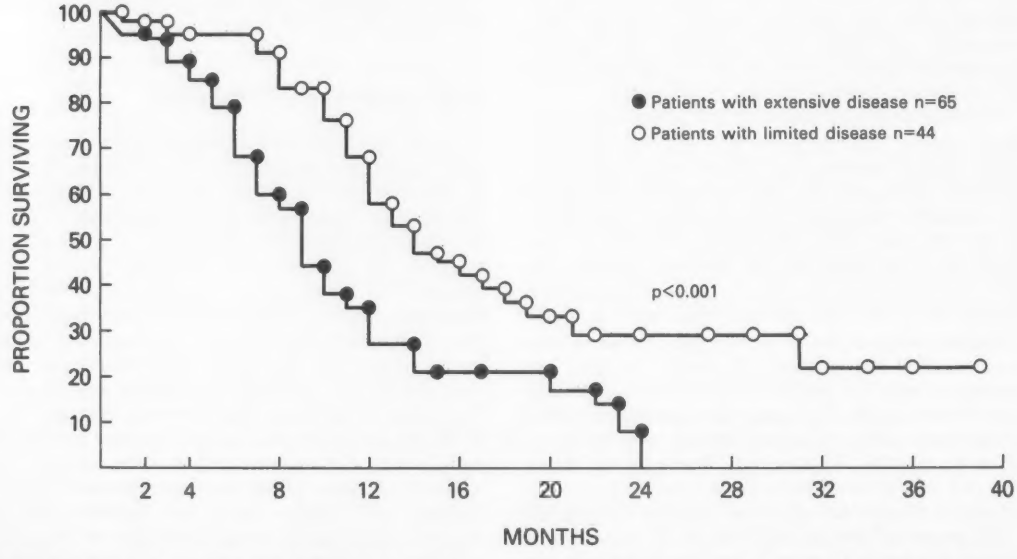


FIGURE 3.—Projected survival for all (109) treated patients according to extent of disease. Patients with limited disease have a median survival of 14 mos compared to 9 mos for patients with extensive disease. These projected curves are virtually identical to the final curves of our earlier study (6). All patients with limited disease who finished 24 mos of therapy have remained free of systemic disease, but one patient who did not receive cranial irradiation died of brain metastases at 30 mos.

diation are considered together with the 14 who were randomized not to receive cranial irradiation, the comparison is also significant ($P < 0.008$). Because of the apparent benefit seen in the interim analysis, randomization to prophylactic cranial irradiation was stopped and all patients who subsequently achieved CR received prophylactic whole-brain irradiation in ten fractions. Thus far, among the 54 patients who achieved CR, four presented with brain metastases, 25 either refused irradiation or were randomized to observation, and 25 have received prophylactic whole-brain irradiation. So far, only one of the 25 irradiated patients has developed a brain metastasis, and the comparison of incidence of brain metastases in the total groups (one of 25 vs nine of 25) is also significant ($P = 0.005$). One patient who received prophylactic cranial irradiation relapsed systemically and then developed leptomeningeal metastases 3 months later. When the patients who developed cranial metastases are censored from the survival curves, this produces no change in the survival curves or median values. Similarly, there was no statistically significant benefit in survival for patients who achieved CR.

Leptomeningeal carcinomatosis was documented in 15 patients, only one of whom received prophylactic cranial irradiation; it was suspected in an additional two patients but could not be confirmed by either cytology (lumbar puncture refused by one patient) or necropsy (refused by both). Thirteen of the patients had extensive disease at presentation and two had limited disease. The cytologic, clinical, pathologic, and therapeutic aspects of leptomeningeal carcinomatosis in these patients have been previously described (27,28).

## Toxicity

The toxicity from this therapy was moderate. Most blood cell count decreases and infectious complications occurred during the first few courses of treatment and were later largely avoided by reducing doses, primarily of cyclophosphamide and doxorubicin, except in those patients with documented bone bone marrow invasion with tumor in which case no dose adjustment was made. Table 4 shows the lowest median wbc and platelet count nadirs (for all courses) according to treatment regimen. The major hematologic toxic effect was leukopenia, principally in the early courses; wbc count nadir ranged from 100 to 2300/μl. In general, wbc suppression was similar for both regimens but tended to be slightly more severe in patients with extensive disease. Platelet counts were less severely affected. There were 31 febrile episodes during periods of granulocytopenia, of which 21 were associated with clinically or microbiologically documented infections. The most frequent site of infection was the lower respiratory tract. The most severe toxic effects occurred in patients with the lowest performance status. There were four drug-related deaths in this study, and all occurred in patients with a pretreatment performance status of 3 or 4. Nonhematologic toxicity was evenly distributed between the treatment regimens and included alopecia (all patients); nausea and vomiting, principally on the Day 1 after the cyclophosphamide infusion (about 66% of the patients); and mild mucositis (approximately 15%). Congestive heart failure was seen in only four patients, none of whom had received more than the usually recommended doxorubicin cumulative dose. Diarrhea and constipation were more common in regimen II, and isolated cases of muscle weakness and hallucination were also seen with the alternating regimen, presumably due to the procarbazine. Overall, all the toxic effects were easily managed by dose reduction, patient assurance, and symptomatic treatment. No patient declined chemotherapy because of side effects.

TABLE 4.—Toxic effects of treatment with regimen I or regimen II

| | Limited disease | | Extensive disease | |
|---|---|---|---|---|
| | Regimen I | Regimen II | Regimen I | Regimen II |
| Wbc count nadirs (cells × $10^3$/μl) | | | | |
| Median | 1.2 | 1.0 | 0.5 | 0.8 |
| Range | 0.5–2.1 | 0.1–2.1 | 0.1–2.3 | 0.1–2.0 |
| Platelet count nadirs (cells × $10^3$/μl) | | | | |
| Median | 110 | 65 | 65 | 50 |
| Range | 20–305 | 18–230 | 9–264 | 9–288 |
| No. of febrile episodes during neutropenia (infectious) | 3 (1) | 4 (2) | 11 (9)* | 13 (9)† |

*Includes 3 toxic deaths.
†Includes 1 toxic death.

## DISCUSSION

Small cell carcinoma of the lung is a virulent disease with a rapid onset, and untreated patients have a median survival of 6–8 weeks (1,5,21). Radiotherapy and combination chemotherapy have significant activity against this neoplasm (1,5,21). The application of chemotherapy together with radiotherapy has produced a marked improvement in median survival and occasional long-term survivors (1,3,5,7–9,11–14). Prospectively randomized trials have, however, raised questions about the role of adjuvant radiotherapy in extensive disease (15,25) and other trials suggest that chemotherapy alone may be as effective in enhancing survival and yielding long-term survivors among patients with limited disease (6,16,17). The current study using combination chemotherapy alone with CAVP16 without radiotherapy to the areas of local disease demonstrates that this treatment is highly active against small cell carcinoma of the lung. The CR rates for both limited and extensive disease (64% and 40%, respectively), the projected median survival times (16 and 10 months, respectively), and the percent of long-term survivors with limited disease are virtually identical to the final results of our previous study with CAVP16 (6). These results compare favorably with any combination chemotherapy treatment program or combined-modality treatment program (combination chemotherapy and radiotherapy) that has been published or reported (1,3,5,7–17,29–35). These results indicate that aggressive combination chemotherapy alone is a highly effective treatment for small cell carcinoma of the lung regardless of extent of disease. Therefore, complications of radiotherapy that have been observed with combined-modality treatment of this disease, such as radiation esophagitis and acute and chronic pneumonitis (36), can possibly be avoided.

In this study, the addition of a second chemotherapy combination which is non-cross-resistant with the first combination did not improve the response rate, the survival, or the proportion of patients who achieved long-term, disease-free status. Thus, the alternating treatment plan as used in this study proved to have no advantage over the simpler treatment. Most studies with various alternating chemotherapies have yielded similar results, unless a less effective drug combination was used as the primary treatment (29,30,32–34).

This study confirms the findings of other studies (1,3,5,6) that patients who achieve CR have significantly improved survival compared to those patients who do not achieve CR. Furthermore, there was no significant difference in median survival between limited- and extensive-disease patients who achieved CR. Thus, achievement of CR may be a more important prognostic factor than either performance status or extent of disease prior to therapy.

One of the serious complications of small cell lung cancer continues to be CNS metastases (18–24,36). Several prospective studies, both randomized and historically controlled, have shown that the addition of prophylactic cranial irradiation to treat micrometastases within this sanctuary site has been effective in reducing the incidence of, or delaying the clinical onset of, intracranial metastases (18–24). There are, however, some conflicting data (25). There is also no evidence to suggest that the application of prophylactic cranial irradiation has any effect on overall survival of groups of patients although individual patients may have had increased survival as a result of this treatment. Appropriate timing of cranial irradiation may be a factor since patients who have active systemic disease when cranial irradiation is administered might develop metastases in the CNS after completion of the irradiation. Patients who do not achieve CR do not survive long enough to have clinically manifest disease (19). Therefore, the absence of CNS disease in such patients cannot necessarily be attributed to prophylactic therapy. The current study, therefore, randomized only those patients who achieved CR to receive or not receive prophylactic cranial irradiation. The results of this aspect of the study confirm the value of prophylactic cranial irradiation in preventing or delaying the clinical onset of cranial metastases. None of 15 patients who were randomized to receive cranial irradiation developed intracranial metastases and only one of the 25 who received such irradiation (randomized and nonrandomized) developed intracranial metastases. Five of 14 randomized patients who were not irradiated and four of 11 who refused to be randomized, ie, nine of 25 patients who did not receive irradiation, developed cranial metastases. One of these patients with cranial metastases died without clinical or necropsy evidence of disease at any other site. No patients developed cranial metastases in the interval between the initiation of chemotherapy and the achievement of CR. Although cranial irradiation of micrometastases was effective in reducing the incidence of overt intracranial metastases, it was not possible to demonstrate an effect on overall survival of the patients so treated. The one patient who died with only brain metastases may have benefited from such irradiation. The lack of overall effect on survival is likely due to the fact that most patients relapse systemically or both intracranially and systemically and eventually die of widespread disease. With further improvements in long-term survival in the future,

it may be possible to demonstrate a survival advantage for those patients who received prophylactic cranial irradiation. It is noteworthy that 15 patients, only one of whom received prophylactic cranial irradiation, developed leptomeningeal carcinomatosis 3 months after systemic relapse. This complication usually occurred in the setting of generalized recurrent disease and, as has been noted previously (27,28), this is more likely to occur among patients with extensive disease who have not achieved CR and who have had bone marrow or liver involvement (28).

Preliminary data from an ongoing prospective study designed to reduce the incidence of infectious complications with the use of trimethoprim/sulfamethoxazole suggest an advantage for that treatment (26). Furthermore, preliminary analysis has not shown any adverse effects of these agents on the clinical activity of the antineoplastic compounds. Further evaluation of this approach is necessary to determine its ultimate role in the treatment of small cell lung cancer.

The current study shows that aggressive combination chemotherapy combined with cranial irradiation for presumed cerebral micrometastases is an effective treatment modality for small cell carcinoma of the lung, regardless of the extent of the disease. Despite the effectiveness of the current therapeutic modalities to produce responses, most patients develop recurrent disease in multiple sites including sites of original disease, regardless of chemotherapy or radiotherapy. Thus, small cell lung cancer is a systemic disease at presentation and at relapse. Future developments of more effective systemic treatment of this disease are therefore necessary.

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# Hexamethylmelamine Distribution in Patients With Ovarian and Other Pelvic Cancers [1,2]

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## SUMMARY

**The distribution of hexamethylmelamine (HMM) was investigated in surgical biopsies from 12 patients with pelvic cancer who were given an oral dose of 200 mg at 2–24 hours before the operation. The highest concentrations of HMM were found in tissues, such as omentum and subcutaneous tissue, that have a predominant lipidic component. HMM concentrations in the primary tumor were lower than in other tissues and were similar to those in plasma. HMM concentrations in metastases appeared to be higher than in the primary tumor, and in metastases with a diameter < 3 mm, drug levels were two to ten times those in larger ones. Plotting the HMM concentrations versus time, utilizing the data from different patients, the elimination half-lives were found to be 9.05, 8.82, and 8.53 in plasma, tumor, and subcutaneous tissue, respectively.**

[Cancer Treat Rep 66:231–235, 1982]

Hexamethylmelamine (HMM) is an anticancer agent which has shown reproducible activity in some experimental and human tumors (1–3). In ovarian cancer the drug's efficacy is well-documented as a single agent (4) and in multidrug regimens (5,6). Its mechanism of action is not yet known, and in spite of the structural similarity to triethylenemelamine it does not appear to act as an alkylating agent (7) or to have any antifolate activity.

It has not yet been established whether the activity is derived mainly from the parent compound or from some metabolites. It has been shown, however, that HMM per se inhibits $^3$H-TdR uptake by Ehrlich ascites cells (8) or by an ovarian cancer cell line (9) in vitro.

Our group has already investigated HMM concentrations in plasma or ascites of patients with ovarian cancer (10,11), but drug distribution in tumor and metastases, which is likely to be better related to the antitumoral effect, has not yet been elucidated for HMM or, for that matter, for most of the antineoplastic agents. Methodologic limits and objective difficulties in obtaining samples explain why such studies have seldom been performed.

The lack of toxicity of a single dose of almost 150 mg/m$^2$ of HMM, currently used in many protocols, and the availability of a sensitive and specific analytic method (12) made it feasible to study the distribution of HMM in some surgically accessible tissues of patients treated with the drug.

In the present study we measured HMM concentrations in plasma, in neoplastic tissues, and in subcutaneous and omental tissues from 12 patients with pelvic cancer who had surgery at different times after HMM administration.

## MATERIALS AND METHODS

### Patients

Twelve patients with pelvic malignancy who required laparotomy for diagnostic and therapeutic purposes were admitted to this study. Written informed consent was obtained from all 12 patients.

[1]Received Oct 27, 1980; revised June 16, 1981; accepted June 23, 1981.

[2]Supported by Public Health Service grant CA-29043 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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TABLE 1.—Patient characteristics

| Patient No. | Age (yrs) | Weight (kg) | Surgery* | Diagnosis |
|---|---|---|---|---|
| 1 | 43 | 54 | MSOO + LB | Epithelial ovarian cancer |
| 2 | 54 | 51 | THBSOO + LB | Epithelial ovarian cancer |
| 3 | 44 | 68 | BSOO | Epithelial ovarian cancer |
| 4 | 70 | 70 | THBSOO + LB + AP | Granulosa cell tumor |
| 5 | 71 | 53 | MB | Lymphoma |
| 6 | 63 | 60.6 | THSOO + LB + AP | Ovarian teratocarcinoma |
| 7 | 70 | 45.7 | MB | Epithelial ovarian cancer |
| 8 | 41 | 73 | BSOO | Epithelial ovarian cancer |
| 9 | 58 | 68 | BSOO | Epithelial ovarian cancer |
| 10 | 28 | 56.6 | THBSOO + LY | Colonic carcinoma |
| 11 | 74 | 52.6 | BSO | Epithelial ovarian cancer |
| 12 | 58 | 64 | MB | Epithelial ovarian cancer |

*MSOO = monosalpingo-oophorectomy and omentectomy; LB = lymph node biopsies; THBSOO = total hysterectomy, bilateral salpingo-oophorectomy, and omentectomy; BSOO = bilateral salpingo-oophorectomy and omentectomy; AP = appendectomy; MB = multiple biopsies; LY = lymphadenectomy; BSO = bilateral salpingo-oophorectomy.

Table 1 shows their age, weight, type of surgical operation, and diagnosis. All patients had normal liver and renal functions and a performance status > 70%. Except for patient 1, who had received ten courses of melphalan at standard doses (0.2 mg/kg/day for 5 days) up to 1 month before, no other patient had received radiotherapy or chemotherapy.

A single dose of 200 mg of HMM (112–148 mg/m$^2$) was administered orally before the surgery at intervals of 2, 3, 3.16, 3.50, 4.35, 4.50, 8.50, 9, 12.50, 16, 18, and 24 hours. The time taken to remove tissues was always < 30 mins from the beginning of the operation.

## Surgical Operation

The operation was performed according to the standards of oncologic pelvic surgery. During the operation, biopsies were taken and carefully isolated and examined by a pathologist.

## Sample Collection and Analytic Assay

Blood samples were taken from an arm vein before drug administration and again at approximately the same time as biopsies. In patients 1 and 9, blood was also taken from the ovarian vein; plasma HMM concentrations were the same as those in the arm vein samples.

Biopsy specimens were gently washed with 4°C Dulbecco phosphate-buffered saline, were dried on filter paper, and were covered with aluminum foil and stored (like the plasma samples) at –20°C until analysis.

Tissue specimens were homogenized in 0.2 N HCl and spun down at 8000 rpm.

The supernatant phase and plasma were made alkaline (pH 11.5) and extracted with *n*-hexane. To determine HMM, a gas-liquid chromatographic method with a nitrogen detector was applied as previously described (12). The calibration curve was plotted by the addition of HMM to drug-free plasma (1 ml) or mouse liver homogenate (1 g) at concentrations ranging from 0.01 to 2 μg. Under these conditions, recovery was 82%–87% and the detector response was linear. Before starting, the determination of recovery of HMM from mouse liver was checked to see that the recovery was the same as that from human ovarian tumor.

## Pharmacokinetic Analysis

The half-life was calculated by applying the peeling method to the elimination phase, using a Hewlett-Packard Model S910 A computer.

# RESULTS

Table 2 shows the tissue/plasma concentration ratios of HMM in each of the 12 patients. The highest concentrations of HMM were found in fat (eg, subcutaneous tissue and nonmetastasized omentum), where tissue:plasma concentration ratios ranged from 8.58 to 134. When omentum was metastasized (patients 5, 8, and 11), the concentrations of HMM were much lower than in normal omentum. HMM concentrations in the primary tumor were lower than in the other tissues and similar to concentrations in plasma. HMM concentrations in metastases were higher than in primary tumors in five of the six patients evaluated (table 2). Table 3 compares the tissue:plasma HMM concentration ratios in small (diameter < 3 mm) and large (diameter > 3 mm) metastases. Ratios were two to ten times higher in

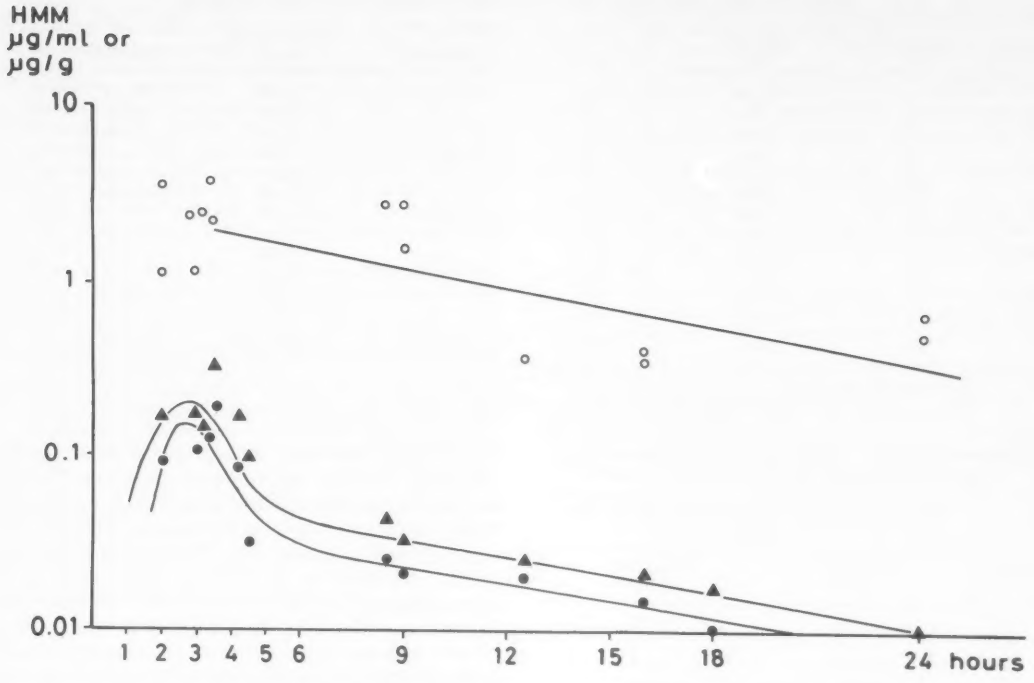


FIGURE 1.—Time course of HMM concentrations in 12 patients with pelvic cancer. ● = plasma; ▲ = tumor; and ○ = fat (subcutaneous). Specimens from all patients + preperitoneal fat from patients 1, 10, and 12.

the small metastases. Drug concentrations were the same in omental and peritoneal metastases. Figure 1 shows the plasma, primary tumor, and subcutaneous concentrations of HMM plotted versus time, using the data obtained in different patients. The pharmacokinetic parameters relative to absorption and distribution could not be calculated because of broad variability among patients. HMM elimination half-life did not differ in plasma (9.05 hours), tumor (8.82 hours), and subcutaneous tissue (8.53 hours).

Plasma and tissue levels of metabolites were not investigated in this study, but pentamethylmelamine, which is extractable under our conditions, never reached 10% of the HMM concentrations.

## DISCUSSION

The anticancer agent HMM appears to be distributed mostly in tissues with a high lipid component (eg, subcutaneous and omental). This was to be expected on the basis of its *n*-octanol water partition coefficient of 350 (13). The drug does not accumulate in fat since it disappears with a half-life of about 9 hours, less than the usual interval of 12 or 24 hours between two subsequent HMM doses.

As previously reported in mice (14), tumoral uptake of HMM does not appear to be selective. In fact, we found the lowest levels in malignant tissue and no appreciable differences between tumors with different biologic features and presumably different sensitivity to HMM.

The concentration of HMM found in tumors in the present study exceeded 0.1 µg/g only in the first hours after drug administration. Thus, the concentrations achieved in the tumor may not be sufficient to ensure a cytotoxic effect. However, this consideration can only be verified by studying the sensitivity of different ovarian cancers, using pharmacokinetically realistic HMM concentrations and times of exposure. The mode of action of HMM is still unknown, so there is, at present, no way of relating the pharmacokinetic data to the drug's activity. Findings reported on the cytotoxic effect of HMM after prolonged exposure of different cell types (9,15)

TABLE 2.—HMM tissue/plasma ratios in patients with pelvic cancer after oral HMM

| Patient No. | Interval (hrs) | Tumor | Metastases | Subcutaneous tissue | Omentum |
|---|---|---|---|---|---|
| 1 | 2.00 | 1.88 | — | 10.90 | 31.01 |
| 2 | 3.00 | 1.66 | 5.00 | 16.55 | 16.36 |
| 3 | 3.16 | 0.92 | — | 29.75 | 68.90 |
| 4 | 3.50 | 1.02 | 5.90 | 8.58 | — |
| 5 | 4.25 | 1.77 | — | 35.77 | 1.44* |
| 6 | 4.50 | 2.75 | — | 63.66 | 134.66 |
| 7 | 8.50 | 4.00 | 9.20 | 101.00 | — |
| 8 | 9.00 | 1.65 | 1.00 | 70.50 | 7.5* |
| 9 | 12.00 | 1.33 | 3.30 | 17.00 | — |
| 10 | 16.00 | 1.43 | 10.01 | 23.33 | — |
| 11 | 18.00 | 1.7 | — | 7.00 | 5.3* |
| 12 | 24.00 | ND† | — | 60 | — |
| Range | 2–24 | 0.92–4 | 1.00–10.01 | 7.00–101.00 | 5.3–134.66 |

*Metastasized omentum.
†ND = not detectable in plasma and tumor (<0.01 μg/ml or g).

TABLE 3.—Comparison of tissue/plasma concentration ratios of HMM in metastases* with diameter < or > 3 mm

| Patient No. | Tissue (μg/g)/plasma (μg/ml) HMM concentration ratio | |
|---|---|---|
| | Metastases < 3 mm | Metastases > 3 mm |
| 3 | 13.223 | 2.396 |
| 4 | 6.565 | 3.484 |
| 10a | 16.200 | 1.533 |
| 10b | 24.333 | 4.866 |

*Omental metastases (patients 3, 4, and 10a); peritoneal metastases (patient 10b).

do not necessarily mean that the unchanged drug is responsible for the cytocidal effect. There is, in fact, recent evidence that tumoral cell line L51784 grown in vitro biotransforms HMM (16), and more recently, murine ovarian cancer was reported to metabolize HMM to chemical species which covalently bind to proteins (17). The concentration we found in the human tumors is likely to be related to the antitumoral activity if HMM acts per se or through activation by tumoral enzymes. However, if the antitumoral activity is mediated by cytotoxic metabolites formed in the liver (eg, methylpentamethylmelamine, a major in vitro metabolite of HMM [18]) and transported to the tumor via the bloodstream, the concentration found in the tumor could be meaningless.

A marked difference was found between HMM concentrations in the primary tumor and in metastases. In animal models, other anticancer agents have already been described as reaching higher concentrations in metastases than in the primary tumor (19). The present study shows that this preferential distribution can also occur in man. One explanation might be that metastases have a better blood supply than the primary tumor, which usually has some ischemic or even necrotic areas. The difference in vascular efficiency might also explain the observation that small metastases concentrate the drug more than larger ones. The higher HMM concentration found in metastases, particularly small ones, suggests that if levels of HMM correlate with its cytotoxic effect, the drug might prove more useful when employed as an adjuvant to surgery to eradicate minimal metastatic foci. Even though the time course was obtained by combining the findings in 12 patients, each tested at a different time after HMM administration, a relatively good fit of the data was observed. Leaving out the earlier phases of absorption and distribution, the elimination rate could be calculated. The plasma elimination half-life of 9 hours was only slightly longer than that previously described: 6.9 ± 0.57 (10). The half-lives and HMM concentrations are similar in plasma and tumor, suggesting that plasma level monitoring may give a good picture of the drug concentration achieved in the tumoral target.

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# Cisplatin, BCNU, Cyclophosphamide, and Prednisone in Multiple Myeloma[1,2]

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## SUMMARY

**Using the MOPC 104E murine plasmacytoma model, Ghanta et al have shown major synergism when cisplatin was added to a treatment program with BCNU and cyclophosphamide (CTX). To evaluate possible synergism in the treatment of human multiple myeloma (MM), 23 evaluable patients who had relapsed with standard treatment were treated with cisplatin, BCNU, CTX, and prednisone. There were five good and four fair responses in this group of heavily pretreated patients. These included responses in four patients who had failed to respond to or had relapsed with BCNU, CTX, and prednisone therapy at higher doses than those used in this protocol, indicating that there is synergism from the addition of cisplatin in the treatment of human MM. Gastrointestinal toxicity was significant but acceptable. Transient renal and hematologic toxicity was also seen. The number of responses and the apparent synergism seen suggest that this combination of drugs should be studied as therapy for untreated patients with MM, especially those with poor prognostic features.**

[Cancer Treat Rep 66:237–242, 1982]

Current treatment programs for multiple myeloma (MM) produce response rates in the range of 45%–60% (1–5). Drugs used in these protocols include melphalan (MEL), cyclophosphamide (CTX), BCNU, and prednisone (PRD). In an attempt to develop a better induction treatment program, the MM Committee of the Southeastern Cancer Study Group (SECSG) has studied a combination of cisplatin, BCNU, CTX, and PRD in patients with MM who have relapsed with, or failed to respond to, standard treatment programs. This combination of drugs was selected on the basis of the studies of Ghanta et al (6) in murine myeloma MOPC 104E. In these studies, the addition of cisplatin to BCNU and CTX produced significant synergism. Low doses of the drugs, which were noncurative as single agents

[1]Received Feb 4, 1981; revised July 20, 1981; accepted July 28, 1981.

[2]Supported by the following Public Health Service grants from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services: CA-03013 (University of Alabama School of Medicine, Birmingham), CA-15584 (Case Western Reserve University School of Medicine, Cleveland, OH), CA-03177 (Duke University School of Medicine, Durham, NC), CA-05634 (Veterans Administration Hospital, Durham, NC), CA-12283 (University of Kentucky School of Medicine, Lexington), CA-12223 (University of Puerto Rico School of Medicine, San Juan), CA-12640 (Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL), and CA-13237 (University of Tennessee Memorial Research Center, Knoxville).

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TABLE 1.—Response to cisplatin, BCNU, CTX, and PRD in the treatment of human MM

| Patient No. | Sex/age (yrs) | Prior treatment* | Results of prior treatment | Interval from diagnosis to start of protocol (mos) | Serum abnormal protein (g/dl) | | | Duration of treatment (mos) from start of protocol | | Results |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | At diagnosis | At start of protocol | At response | To response | To relapse | |
| | | | | | | ***Good response*** | | | | |
| 9 | M/52 | BCP, Rad | Good | 7 | 5.6 | 3.5 | 0.9 | 4 | 14+ | Continuing response 14 mos after starting protocol; off protocol after 9 cycles of treatment due to GI toxicity |
| 14 | M/76 | MP | Good | 20 | 2.5 | 2.5 | 0.7 | 5 | 25 | Continuing other chemotherapy; off protocol after 9 cycles of treatment due to GI toxicity |
| 16 | F/68 | MP, Rad | Good | 37 | 3.0 | 4.2 | 1.1 | 8 | 16 | Continuing other chemotherapy; off protocol after 9 cycles of treatment due to GI toxicity |
| 20 | F/64 | MP | Good | 24 | | 4.1 | 1.8 | 7 | 16+ | Continuing response 16 mos after starting protocol; off protocol after 8 cycles of treatment due to GI toxicity |
| 23 | F/56 | BCP, MP | None, none | 5 | 8.0 | 12.3 | 5.7 | 5 | 7 | Died 10 mos after starting protocol |
| | | | | | | ***Fair response*** | | | | |
| 4 | M/55 | MP, H | Good | 51 | | 5.0 | 2.9 | 8 | 8 | Progression of plasmacytoma after 8 mos of therapy |
| 10 | F/66 | BCP | Fair | 14 | 6.8 | 8.8 | 5.8 | 5 | 6 | Died 6 mos after starting protocol |
| 15 | M/64 | BCP | None | 6 | 4.5 | 4.3 | 3.0 | 2 | 4 | Died 7 mos after starting protocol |
| 21 | M/66 | MCP, B, Ad | Good | 116 | | 4.2 | 2.5 | 5 | 7 | Died 17 mos after starting protocol |
| | | | | | | ***No response*** | | | | |
| 1 | M/54 | MP | None | 10 | 8.5 | 5.6 | 4.7 | | 5 | Persistent bone pain |
| 2 | F/68 | BCP, MP | Fair | 16 | 4.9 | 2.2 | 3.0 | | 4 | Stable disease, 5+ mos |
| 3 | M/49 | BCP | None | 6 | 2.7 | 1.9 | 1.3 | | 4 | Persistent bone pain |
| 5 | M/75 | MP, CP, Rad | Good | 65 | | 10.5 | 6.7 | | 1 | Off study due to renal toxicity; stable disease 4 mos later |
| 6 | F/74 | CP, Rad | Good | 132 | | 4.3 | 0.7 | | 3 | CTX cystitis, bleeding, died of sepsis 1 mo later; wbc count normal |
| 7 | M/49 | BCP, MeP | Good | 64 | | 0.9 | 1.0 | | 5 | Refused further therapy after 5 cycles due to GI toxicity; died 17 mos after starting protocol |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 8 | M/49 | BCP, MEL, H | Good | 92 | | 8.0 | 6.5 | 2 | Increasing bone pain; developed acute myelocytic leukemia |
| 11 | M/73 | BCP, HP | Good | 25 | | 8.8 | 8.0 | 2 | Died 6 mos after starting protocol |
| 12 | F/60 | MP, BCP, Mi | Good | 12 | 1.4 | 2.2 | 3.9 | 3 | Progression with hypercalcemia; died 3 mos after starting protocol |
| 13 | M/41 | BCP, MP | Fair | 14 | 4.3 | 4.3 | 3.7 | 4 | Stable disease |
| 17 | M/65 | MP | Good | 30 | 2.8 | 3.3 | 3.4 | 3 | Died 5 mos after starting protocol |
| 18 | F/49 | BCP, MP | Good | 55 | 2.3 | 3.8 | 3.0 | 1 | Progression with hypercalcemia; died 3 mos after starting protocol |
| 19 | M/65 | MP, CP | Good | 58 | | 2.6† | 2.1† | 3 | Progression with hypercalcemia; died 6 mos after starting protocol |
| 22 | M/58 | BCP, Ad, V, MP | Fair | 6 | 6.8 | 1.1 | | 1 | Progression of plasmacytomas; died 5 mos after starting protocol |

*BCP = BCNU + CTX + PRD; Rad = local radiotherapy; MP = MEL + PRD; H = hexamethylmelamine; MCP = MEL + CTX + PRD; B = BCNU; Ad = doxorubicin; CP = CTX + PRD; MeP = methyl-CCNU + PRD; HP = hexamethylmelamine + PRD; Mi = mithramycin; V = vincristine.
†Urinary Bence Jones protein (g/24 hrs).

or in the combination of BCNU and CTX, produced 100% responses and 80% survivals when given to the mice as a three-drug combination. This murine plasmacytoma has also proven to be an excellent model for screening of drugs which are active in human MM (7,8). Twenty-three evaluable patients were treated with the combination of cisplatin, BCNU, CTX, and PRD, and the results are reported here.

## METHODS

Patients with MM whose disease was progressing despite chemotherapy, or who had failed to respond to standard protocols, were eligible for this protocol if their creatinine clearance was > 50 ml/minute and if they gave informed consent. Eligible patients included those who had failed with protocols containing BCNU, CTX, and PRD, since we were looking for potential synergism from the addition of cisplatin to this combination of drugs. Four patients had failed to respond to their initial therapy for MM. Three of these patients (Nos. 3, 15, and 23) had been nonresponders to a protocol using BCNU, CTX, and PRD, and two (patients 1 and 23) had failed to respond to MEL and PRD. The remaining patients had relapsed after initial responses to a variety of treatment programs as indicated in table 1.

Drugs were given at 4-week intervals unless renal or bone marrow toxicity prevented treatment. A course of treatment consisted of cisplatin (50 mg/m$^2$), BCNU (50 mg/m$^2$), and CTX (300 mg/m$^2$) iv on Day 1 and PRD (75 mg/day orally) for 7 days. Furosemide at a dose of 20 mg orally and a total fluid intake > 3 liters were given during the first 24 hours to ameliorate the renal toxicity of cisplatin.

Patients were evaluable for response after two or more cycles of treatment or after one cycle if there was obvious progression of disease (ie, increase in the size of plasmacytomas), new lesions, or excessive toxic effects. A good response was defined as a > 50% reduction in the abnormal protein with a 50% improvement of an anemic (Hgb < 8 g/100 ml) pretreatment hemoglobin level over a 1-month period or maintenance of a hemoglobin level > 9 g/100 ml, without transfusions. A fair response was defined as a > 25% but < 50% reduction in the abnormal protein. Responding patients continued to receive treatment until disease progression or until the patient refused further therapy. Nonresponders or those with obvious progression of disease were removed from the study.

### Toxicity

The criteria for bone marrow toxicity were: *hemoglobin, grade I*—> 3-g decline from baseline, and *grade II*—> 5-g decline from baseline; *wbc count, grade I*—> 1500/mm$^3$ decline from baseline > 3000/mm$^3$ or decrease to < 750/mm$^3$ from baseline < 3000/mm$^3$, and *grade II*—decline to < 750/mm$^3$ from baseline > 3000/mm$^3$ or to < 200/mm$^3$ from baseline < 3000/mm$^3$; *platelet count, grade I*—> 50,000/mm$^3$ decline from baseline < 130,000/mm$^3$ or decrease to < 100,000/mm$^3$ from baseline > 130,000/mm$^3$, *grade II*—> 80,000/mm$^3$ decline from baseline < 130,000/mm$^3$ or decrease to < 50,000/mm$^3$ from baseline > 130,000/mm$^3$, and *grade III*—grade II toxicity with life-threatening hemorrhage. The criteria for GI toxicity were: *grade I*—mild nausea and vomiting controlled by antiemetics; and *grade II*—nausea and vomiting not controlled by antiemetics. The criteria for renal toxicity were: *grade I*—transient increase in serum creatinine to > 1.2 but < 2.0 mg/100 ml and transient decrease in creatinine clearance to < 60 but > 50 ml/minute; *grade II*—transient increase in serum creatinine to > 2.0 mg/100 ml and transient decrease in creatinine clearance to < 50 ml/minute; and *grade III*—persistent renal failure with serum creatinine > 3.0 mg/100 ml and/or creatinine clearance < 20 ml/minute.

## RESULTS

Twenty-three evaluable patients were treated. Information concerning those patients who showed a good or fair response is shown in table 1. Two patients had progression of their disease after one cycle of therapy (one died of refractory hypercalcemia and the other had progression of multiple plasmacytomas). In one patient, the protocol was discontinued after one cycle of therapy because of renal toxic effects. Twenty patients received two or more cycles of therapy; five patients achieved a good response, four patients had a fair response, and the remaining patients had no response, by protein measurement, or had progressive disease while receiving treatment. One patient was considered to be a nonresponder in spite of a > 50% decline in the abnormal protein. This patient died from sepsis 5 weeks after her third cycle of treatment, with a normal wbc count and bleeding due to thrombocytopenia.

Including all of the responders, the median duration of response from the beginning of therapy to relapse was 9 months (range, 3–28). Seven of the nine responding patients had responses lasting ⩾ 7 months. These included all of the good responders and two of the fair responders.

The responding patients showed a definite survival advantage over the nonresponders. Four patients (one with a good response and three with fair responses) had died a median of 9 months after

starting cisplatin therapy. Five other responding patients were still alive, with a median survival of 16 months after the start of the cisplatin protocol; two had continuing good responses, and three had relapsed (two with a good response and one with a fair response) but were continuing to receive other therapy. Of the nonresponders, five were alive 4–5+ months after starting cisplatin therapy. Eight nonresponding patients had died a median of 5 months after entry in this protocol.

There was significant toxicity from the treatment program as shown in table 2. Hematologic toxicity was frequent but caused only brief delays in therapy. Twelve patients required rbc transfusions, but in several of these patients it appeared that the need for transfusions related to the underlying disease and not the therapy. The transient leukopenia which occurred was not a severe problem. The patient who died of sepsis had a normal wbc count when the sepsis developed. Thrombocytopenia was common but, with the exception of the patient mentioned previously, was not a life-threatening problem.

Renal toxicity was frequent but not severe enough to prevent adequate therapy. Ten patients had increases in serum creatinine and/or decreases in creatinine clearance, which reverted to previous levels after a short time.

The major toxicity was gastrointestinal, with 21 patients having nausea and vomiting. In two thirds of these patients, standard antiemetic therapy did not control the vomiting. Nevertheless, this toxic effect did not prevent the patients who responded from receiving adequate induction treatment. Ultimately, five patients refused treatment because of the severe GI toxic effects. One patient with stable disease received five courses but there was no response of his abnormal protein, and another had a fair response of his protein but declined further therapy after eight courses. The remaining three patients had good responses but refused to continue therapy after nine, nine, and ten courses. The latter patient is continuing therapy with BCNU, CTX, and PRD, another patient was followed with no treatment and relapsed after 20 months, and the remaining patient has maintained a good response after 3 months without the treatment.

TABLE 2.—Toxicity*

| | Grade†— | | |
|---|---|---|---|
| | I | II | III |
| Rbc count | 7 | 5 | |
| Wbc count | 8 | 1 | |
| Platelet count | 10 | 2 | 1 |
| GI | 8 | 13‡ | |
| Renal | 5 | 6 | |
| Genitourinary | | 1§ | |
| Skin | 1 | | |

*Values = No. of patients.

†*See* text for grading of toxicity.

‡5 patients refused further treatment due to GI toxicity after multiple courses of therapy.

§CTX cystitis.

## DISCUSSION

The treatment protocol used in these studies was chosen on the basis of the work of Hiramoto et al (7,8), which showed that the MOPC 104E murine myeloma was an excellent screening model capable of detecting agents effective in the treatment of human MM, as well as identifying some which are not efficacious. Efficacy was shown by a major decrease in the tumor-associated IgM and by disease-free survival of the treated animals. Using this system, Ghanta et al (6) showed that low doses of BCNU, CTX, or cisplatin given as single agents were ineffective in treating the MOPC 104E myeloma model, with < 10% minimal responses and no survivors. Using the same doses of BCNU and CTX as a two-drug combination produced a minimal transient response of the idiotypic IgM and no survivors at 100 days. The addition of the same ineffective low dose of cisplatin to the low-dose BCNU and CTX combination was strikingly effective, producing 100% major responses as shown by the IgM levels and 80% long-term disease-free survival. Thus, there appeared to be major synergism achieved by the addition of cisplatin to the BCNU and CTX.

The studies reported here were an attempt to extend these findings to the treatment of human MM. The doses of BCNU and CTX were somewhat lower than those used in an SECSG protocol, which produced a response in 53% of the previously untreated MM patients (9). There is no background of data to provide a basis for choosing a dose of cisplatin which might be effective in treating human myeloma. The dose of cisplatin used in these studies is lower than the doses used to treat most solid tumors.

In spite of these relatively low doses of all the drugs, the combination seemed to be effective, producing a response rate (good and fair) of 45% in a group of heavily pretreated patients who were in relapse or had failed to respond to induction therapy. It was of special interest that two patients (No. 23, good response and No. 15, fair response) had failed to respond to induction treatment on a protocol using higher doses of BCNU and CTX with PRD. Two other patients (No. 9, good response and No. 21, fair response) had relapsed after having had a good and a fair response, respectively, to the higher

dose of BCNU, CTX, and PRD. These results support the possibility of synergism in the treatment of human MM from the addition of cisplatin to the three-drug combination, as was suggested by the animal studies.

Only one other group of patients with alkylating-agent-resistant MM that was treated with a cisplatin-containing protocol has been reported (10). The Southwest Oncology Group (SWOG) treated 16 heavily pretreated patients with bleomycin, vincristine, BCNU, doxorubicin, and PRD on Day 1, followed by cisplatin at a dose of 20 mg/m²/day on Days 2–5 every 3 weeks. They report 25% good responses (> 50% decrease in abnormal protein). They did not comment on the toxicity of the treatment. They state that the program did not appear to be better than other second-line protocols for the treatment of MM.

The response rate for our SECSG protocol is about the same in terms of good responses as that reported by SWOG. The SWOG report does not mention less than good responses. Since we did see fair responses in four additional patients and also obtained responses in patients who had failed on higher doses of the same alkylating agents, we consider that this protocol does have advantages over other second-line treatment programs.

The major problem resulting from the addition of cisplatin to the treatment program in this protocol was the GI toxicity. Currently available antiemetic regimens did not control nausea and vomiting, which was almost universal and frequently severe. Patients were able to tolerate this treatment for a limited number of cycles, but prolongation of the program led to progressive dropping out of patients even though they were achieving a good response.

Other toxic effects from the therapy were less of a problem. Hematologic toxicity was mostly of a low grade and did not appear to be worse than that seen with other treatment programs for patients with myeloma at this stage of their disease. Similarly, renal toxicity, while common, was generally not severe. A major concern in developing this protocol had been that patients with myeloma, who have many factors predisposing to poor renal function, might not be able to tolerate the added renal insult from cisplatin. This did not prove to be the case. The renal functional abnormalities which were seen were transient, and only one patient was removed from the study for this reason.

It appears from this study that the combination of cisplatin, BCNU, CTX, and PRD in the doses used is an effective treatment for MM, even in advanced cases. The addition of cisplatin appears to be the major factor in producing the severe GI and mild renal toxicity seen, since neither of these was a major problem in SECSG protocols using BCNU, CTX, and PRD. On the other hand, the fact that this protocol appears to have produced responses in patients who earlier had failed on higher doses of BCNU and CTX with PRD suggests that the addition of cisplatin may be significant in enhancing the effectiveness of the treatment. This should be evaluated further in patients at earlier stages of their disease, especially in those patients in high-risk categories.

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# Effective Control of Cisplatin-Induced Nausea Using High-Dose Steroids and Droperidol [1]

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## SUMMARY

**Seventy-three consecutive patients receiving cisplatin-containing chemotherapy regimens were treated with an initial dose of chlorpromazine (25 mg im), droperidol (1 mg iv, then 1 mg sc every 4 hours), and methylprednisolone (250 mg every 4 hours for four doses). Twenty patients received a combination chemotherapy protocol that included cisplatin at a dose of 40 mg/m² iv (group 1) and 53 patients received a protocol that included cisplatin at a dose of 120 mg/m² iv (group 2). The median age of all the patients was 58 years. Seventy-five percent of the patients in group 1 and 45% of those in group 2 had no vomiting at all; the remainder had limited and tolerable vomiting. These results in a group of elderly patients compare favorably with trials of other antiemetic programs.**

[Cancer Treat Rep 66:243–245, 1982]

Nausea and vomiting secondary to administration of the antineoplastic drug, cisplatin, occurs in nearly 100% of the patients, is often intractable to standard antiemetics, and may demoralize both patients and their physicians (1). In a recent report on the use of cisplatin in combination chemotherapy protocols for a variety of tumors, 6% of the patients refused further treatment because of acute gastrointestinal toxic effects (2).

We have developed an empiric program of antiemesis which effectively limits the acute gastrointestinal toxicity of cisplatin. High doses of the corticosteroid, methylprednisolone (3), were administered iv in combination with small parenteral doses of the butyrophenone drug, droperidol (4). No serious adverse effects resulting from this program were detected. The prevention of nausea and vomiting was most gratifying in an elderly group of patients.

## PATIENTS AND METHODS

Seventy-three consecutive patients were treated with two regimens of systemic chemotherapy containing cisplatin for metastatic non-small cell lung carcinoma. Their ages ranged from 41 to 77 years (median, 58). The mean Eastern Cooperative Oncology Group performance status was 1.7 (range, 0–4). Twenty patients (group 1) received a 1-hour infusion of cisplatin at a dose of 40 mg/m² iv combined with mitomycin at a dose of 10 mg/m² iv and vinblastine at a dose of 6 mg/m² iv. Fifty-three patients (group 2) received a 2-hour infusion of cisplatin at a dose of 120 mg/m² iv combined with mitomycin at a dose of 10 mg/m² iv, vinblastine at a dose of 6 mg/m² iv, and bleomycin at a dose of 5–15 mg iv.

The antiemesis program consisted of chlorpromazine at a dose of 25 mg im, droperidol at a dose of 1 mg iv just prior to chemotherapy and then 1 mg sc every 4 hours until the patient was asleep, and methylprednisolone at a dose of 250 mg iv just

[1]Received Feb 12, 1981; revised June 15, 1981; accepted June 25, 1981.

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[4]We thank Ruth Peter, RN, Dorothy Rossi, and Florence Ashenbrenner for their invaluable assistance in reviewing the patient records for followup information, and Kris Aiena for typing the manuscript.

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before initiation of chemotherapy and then every 4 hours to a total of 1 g over 16 hours. If a patient began to vomit, he was given additional chlorpromazine as needed every 4 hours.

Patients were evaluated for emesis during their first course of chemotherapy only. All patients were hospitalized. Patients in group 1 were admitted for 24 hours and were given furosemide and 1 liter of iv fluid prior to chemotherapy. Patients in group 2 were hydrated overnight, were given furosemide, and were infused with 2 liters of ½ normal saline followed by an infusion of mannitol and an additional 1 liter of ½ normal saline. The nursing service evaluation of time and quantity of emesis and requirements for additional antiemetic therapy was reviewed on the day of discharge from the hospital. In addition, two investigators (B. A. Mason and J. Dambra) visited all patients at least once during the afternoon of chemotherapy administration and interviewed them on the day of discharge from the hospital. The amount of nausea and vomiting in temporal relationship to the chemotherapy administration was recorded. Nausea was categorized as absent, persistent (lasting > 60 minutes), or limited (lasting < 60 minutes and then resolving for the duration of the treatment day).

## RESULTS

Thirty-nine patients had no nausea or vomiting at all (53%), 33 had limited nausea and vomiting, and one had persistent vomiting for 7 hours. Fifteen of 20 patients in group 1 had no vomiting (75%) and the remainder experienced limited vomiting. In group 2, 24 patients had no vomiting (45%), 28 experienced limited vomiting, and one had persistent vomiting. The difference in absolute control of vomiting was statistically significant for the two dose levels of cisplatin ($P < 0.05$ by the chi-square method). Most patients who were in the "limited vomiting" category were symptomatic for a short time at the conclusion of the cisplatin infusion. All patients experienced mild somnolence (easily aroused) during the 4–6 hours after chemotherapy was initiated. With few exceptions, the patients were awake and alert in the late afternoon, continued receiving the droperidol until their usual bedtime, and slept through the night. Sixty-two of the patients felt well the morning after chemotherapy and were able to eat breakfast with a hearty appetite. Interestingly, many of these patients felt their best on the morning after chemotherapy administration (probably due to the methylprednisolone) and the temporary relief from the chronic malaise of the malignancy or its therapy was often dramatic. The remaining 11 patients (including ten in group 2) had mild, but not disabling, symptoms of nausea on the morning after chemotherapy.

Delayed chronic nausea and anorexia did occur in 12 patients (ten patients in group 2) subsequent to their discharge from the hospital and eight patients required readmission for treatment of intractable nausea (seven patients in group 2). No patient wished to discontinue the chemotherapy program because of acute gastrointestinal toxic effects.

Although the study design eliminated anticipatory nausea primarily by analyzing the pattern of nausea during the first course of chemotherapy, followup data are available for all the patients. Nine patients in group 1 who experienced no vomiting during the first cycle of chemotherapy and received more than one course were also protected from vomiting during 79% of a total of 29 subsequent courses of therapy. Fourteen patients in group 2 were protected from vomiting in 77% of 44 subsequent cycles of chemotherapy. The patients who "failed" during their second course of chemotherapy had only minimal vomiting. Five of these patients reported complete control of nausea and vomiting again during subsequent courses of chemotherapy.

## DISCUSSION

Cisplatin induces nausea and vomiting in almost all patients and persistent intractable vomiting in many patients (1). Standard phenothiazine antiemetics have not been successful in controlling this toxic effect. Numerous agents have been tried to control chemotherapy-induced nausea and vomiting. The most promising agents in recent years have been delta-9-tetrahydrocannabinol (THC) (5–10), nabilone (11), a synthetic cannabinoid, metoclopramide (12), high-dose corticosteroids (3,13), and droperidol (4,14–17).

Droperidol, a butyrophenone derivative, has been used as a prophylactic antiemetic agent for minor surgical procedures (14). It has been effective in preventing and treating postoperative nausea and vomiting after balanced general anesthesia as well (15,16). Grossman et al (4) suggested that droperidol might be effective in preventing cisplatin-induced nausea and vomiting. A recent small controlled study showed droperidol to be more effective than prochlorperazine in ameliorating the nausea caused by cisplatin-containing chemotherapy regimens (17). Recent reports of the antiemetic effects of high-dose steroids have also appeared (3,13).

Our study was an outgrowth of earlier attempts at The Fox Chase Cancer Center to control the intractable nausea and vomiting associated with cisplatin. Droperidol and high doses of methylprednisolone were combined after some preliminary ex-

perience with each agent alone. The initial single dose of chlorpromazine is a holdover from earlier antiemetic programs. All patients with lung cancer consecutively tr[illegible]d with cisplatin-containing chemotherapy reg[illegible] were included in this uncontrolled study.

The results were impressive and compare favorably with published reports concerning THC and nabilone. Our patients had a higher median age and a much lower incidence of toxic effects from the antiemetic drugs themselves. Complete abolition of vomiting was more successful in group 1. However, we did not observe the kind of persistent intractable vomiting lasting many hours in any patient, a marked contrast to our previous experience. Most episodes of vomiting occurred just after the cisplatin was infused and were short-lived. The patients did not experience nausea later in the day and almost invariably enjoyed a restful night's sleep. No patient dreaded a future course of chemotherapy because of past experience.

High-dose methylprednisolone combined with low doses of droperidol effectively limits cisplatin-induced nausea in this uncontrolled trial. The euphoric effect of methylprednisolone appears to be beneficial for patients during the day after chemotherapy. This program has the advantages of low toxicity in an elderly group of patients and availability of both drugs at this time to all medical oncologists. A controlled trial is necessary to confirm the efficacy of this antiemetic program.

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# Cisplatin, Doxorubicin, Cyclophosphamide, and Vindesine Combination Chemotherapy for Non-Small Cell Lung Cancer[1,2,3]

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## SUMMARY

**Seventy-four patients with non-small cell lung cancer were treated in a prospective, randomized trial either with a four-drug combination of cisplatin, doxorubicin, cyclophosphamide, and vindesine (PACE) or with a three-drug combination of cisplatin, cyclophosphamide, and vindesine (PCE). None of these patients had received prior chemotherapy, and all had a Karnofsky performance status of at least 60. Of 68 evaluable patients, 21 (31%) had complete or partial remissions. Response rates for PACE and PCE were similar, and there was no difference in response rates for patients with adenocarcinoma or epidermoid cancer. The median duration of remission was 10 months (range, 2–26+); five patients are still in remission (median, 18+ months; range, 17+ to 26+). The median duration of survival for responding patients (complete or partial) was 18 months. Toxic effects, including mild to moderate myelosuppression, peripheral neuropathy, and nephrotoxicity, were manageable in general. The response rates and remission durations for PACE and PCE are similar to those seen with the two-drug combination of cisplatin and vindesine, and toxic effects are similar. Thus, the addition of doxorubicin and/or cyclophosphamide adds no advantage to the use of the cisplatin and vindesine combination alone.**

[Cancer Treat Rep 66:247–251, 1982]

Lung cancer remains a major cause of cancer-related deaths in the US. Over 117,000 new cases occur each year; with current survival statistics, > 90% of these patients will die within 5 years of their diagnosis (1). Most patients will either present with or will develop metastatic disease requiring systemic chemotherapy. A variety of conventional agents have been used, either alone or in combination, in the treatment of non-small cell lung cancer (2,3). Although initial response rates in pilot studies may have been encouraging, followup trials were usually more disappointing (3,4). The unsatisfactory results obtained from conventional agents have led to increased interest in new investigational drugs.

Phase II studies with cisplatin and vindesine had indicated the activity of the two drugs used as single agents (5–8). Recently, the two drugs were combined, and in a group of 83 previously untreated patients, a 43% complete and partial response rate was noted (9). Because earlier studies by Eagan et al (10), and subsequently others (11,12), demonstrated the activity of the three-drug combination

[1]Received Feb 12, 1981; revised June 22, 1981; accepted July 22, 1981.

[2]Supported in part by contract N01-CM-07337 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Kelsen is a Landrum/Karnofsky Scholar, and Drs. Gralla and Casper are Junior Faculty Fellows of the American Cancer Society.

[3]Presented in part at the Second International Symposium on Lung Cancer, Copenhagen, Denmark, June 12, 1980.

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of cyclophosphamide, doxorubicin, and cisplatin (CAP), the current study was designed to assess the effects of the addition of doxorubicin and/or cyclophosphamide on the rates and durations of response seen with vindesine and cisplatin.

## MATERIALS AND METHODS

Seventy-four patients with histologically or cytologically proven non-small cell lung cancer were entered in this study from July 1979 to April 1980. The diagnosis was confirmed by the Department of Pathology, Memorial Sloan-Kettering Cancer Center. Patient characteristics are shown in table 1. All patients had a Karnofsky performance status (PS) of at least 60 and measurable disease that had not been treated with radiation; none had received prior chemotherapy. Sites of metastatic disease are shown in table 2. All patients had clinically normal auditory function, a creatinine clearance > 65 ml/minute, and no evidence of congestive heart failure (New York Heart Association class I or II); informed consent was obtained from all patients before start of chemotherapy.

Before entering the study, all patients had a history and physical examination, chest X-ray, cbc with differential, platelet count, 12-channel screening profile, 24-hour creatinine clearance, ECG, and urinalysis. If clinically indicated, audiograms and radionuclide or computerized tomographic (CT) studies were performed. Interval history and physical examinations were performed at least every 2 weeks, and a chest X-ray was done at least once a month. Prior to each course of chemotherapy, a cbc and platelet count were performed; a creatinine clearance was done before each course of cisplatin.

TABLE 1.—Patient characteristics

| Characteristic | |
|---|---|
| No. of patients | |
| Entered | 74 |
| Evaluable | 68 |
| Unevaluable | 6 |
| Sex | |
| Males | 53 |
| Females | 21 |
| Prior radiotherapy (%) | 21 (31) |
| Age (yrs) | |
| Median | 54 |
| Range | 30–66 |
| PS | |
| Median | 70 |
| Range | 60–100 |
| Cell type (% of patients) | |
| Adenocarcinoma | 70 |
| Epidermoid carcinoma | 28 |
| Large cell carcinoma | 2 |

TABLE 2.—Sites and extent of metastatic disease

| | % of patients |
|---|---|
| Site of disease | |
| Lung | 90 |
| Lung (only site) | 16 |
| Liver | 7 |
| Lymph nodes | 38 |
| Subcutaneous tissue | 19 |
| Brain | 8 |
| Bone | 35 |
| Extent of disease* | |
| Limited | 16 |
| Extensive | 84 |

*Veterans Administration Lung Group criteria.

After stratification for cell type (epidermoid carcinoma vs adenocarcinoma) and PS (good, PS of 80–100; fair, PS of 60–70), patients were randomized between one of two multidrug combinations.

The first arm of therapy included cisplatin, doxorubicin, cyclophosphamide, and vindesine (PACE); the second arm included cisplatin, cyclophosphamide, and vindesine (PCE). Drug doses and scheduling are shown in table 3. If doxorubicin was not given, the dose of cyclophosphamide was increased to 500 mg/m$^2$; dose scheduling on the PCE arm was otherwise the same as for PACE. Cisplatin was given with prehydration and mannitol as previously described (13). Attenuation of drug dose was as follows. A full dose of cisplatin was given unless the creatinine clearance decreased to < 60 ml/minute; cisplatin was then withheld until the creatinine clearance increased to > 60 ml/minute. Full doses of doxorubicin, cyclophosphamide, and vindesine were given if the wbc count was > 3000/mm$^3$ and the platelet count was > 100,000/mm$^3$. If the wbc count was > 2000 but < 3000/mm$^3$, or if the platelet count was > 50,000 but < 100,000/mm$^3$, 50% of the calculated dose was given. If the wbc count was < 2000/mm$^3$ and/or the platelet count was < 50,000/mm$^3$, chemotherapy was withheld. The dose of vindesine was halved in the presence of moderate neuropathy and was withheld if severe neuropathy was noted. The total dose of doxorubicin was limited to 450 mg/m$^2$. At that point, doxorubicin was discontinued, and the cyclophosphamide dose was increased to 500 mg/m$^2$.

Clearly definable pulmonary metastases and lymph node or subcutaneous masses were accepted as measurable disease. Hepatomegaly or abnormal liver scans were not considered measurable, but a CT scan of the liver or abdomen revealing clearly measurable lesions was acceptable. Abnormal bone scans

TABLE 3.—PACE chemotherapy for non-small cell lung cancer

| Drug | Day | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 8 | 15 | 22 | 29 | 36 | 43 |
| Cisplatin (120 mg/m²) | x | | | | | | x |
| Doxorubicin (30 mg/m²) | x | | | x | | | x |
| Cyclophosphamide (300 mg/m²) | x | | | x | | | x |
| Vindesine (3 mg/m²) | x | | x | x | | x | x |

or bone X-rays were not considered measurable but were followed. Fifty-seven patients had extensive disease by the Veterans Administration Lung Group criteria; 11 had limited disease. Criteria for response have been described previously (5).

## RESULTS

Seventy-four patients were randomized to receive either PACE (38 patients) or PCE (36 patients). Both groups were similar in cell type, male:female ratio, PS, and age. Seventy percent of the patients had adenocarcinoma; 28% had epidermoid carcinoma; and 2% had large cell undifferentiated carcinoma. Of the six unevaluable patients, three had initially been randomized to receive PACE, and three had been randomized to receive PCE. Five patients were unevaluable because of major protocol violations (no measurable disease), and one patient was lost to followup after receiving one dose of PCE. Among the 68 evaluable patients, four had complete remission (CR) and 17 had partial remission (PR), for an overall major objective response rate of 31%. Of the 21 patients with CR or PR, four (all PRs) had limited disease and 17 had extensive disease. CR and PR were seen in 24% of the patients receiving PACE and in 36% of those receiving PCE. However, this difference was not statistically significant. Two CRs (5%) were seen with PACE, and two (5.5%) were observed with PCE. Responses were seen in all sites of disease, including pulmonary metastases, the primary site, lymph nodes, subcutaneous metastases, and hepatic metastases.

The median duration of response for those patients having CR or PR was 10 months; of the 21 responding patients, 16 have relapsed at 2–15 months. The remaining five patients are in remission at 17+ to 26+ months.

The median duration of survival for all 68 patients was 8 months. There was no difference in survival for those receiving PACE or PCE, for those with adenocarcinoma or epidermoid carcinoma, or for women versus men.

For patients achieving CR or PR, the median duration of survival was 18 months, which was significantly superior to the survival of the remaining patients, who had minor regression, stable disease, and disease progression ($P < 0.01$) (fig 1). Seven patients are still alive. However, even patients with minor regression or stable disease had a survival advantage, compared to those demonstrating tumor progression (8 vs 4 months; $P < 0.01$).

Sixty-eight patients were considered to be evaluable for toxicity. In general, the toxic effects of this program were easily manageable. Nausea and vomiting were seen in all patients and usually were most pronounced when cisplatin was administered. Conventional antiemetics were of only modest benefit in most patients. Alopecia was almost universal and was usually pronounced. Nephrotoxicity, defined as a peak serum creatinine > 2.5 mg/100 ml on any course, was seen in seven (10%) of the patients. In almost all of the patients, it subsequently returned toward normal. Severe ototoxicity was noted in three patients and led to permanent discontinuation of cisplatin therapy. Tinnitus was more common but was not dose-limiting. Approximately one third of the patients had diarrhea after receiving cisplatin.

Paresthesias and numbness were noted in almost all patients receiving at least three or four doses of vindesine, but severe peripheral neuropathies, requiring marked reduction of drug dose or permanent interruption of vindesine therapy, were seen in only five of the 68 patients (7%). Posttreatment myalgias and fever were common (approximately 20%) but were not dose-limiting.

No patient had clinical evidence of doxorubicin cardiotoxicity, and none had hemorrhagic cystitis.

Myelosuppression was moderate: median wbc count nadir of 2600/mm³ (range, 100–13,500) and median platelet count nadir of 198,000/mm³ (range 6000–680,000); however, 15% of the patients had a wbc count nadir of < 1000/mm³ during at least one course of therapy. Four patients required hospitalization for fever during their nadir period. One patient with progressive disease received radiation therapy shortly after receiving chemotherapy and died with leukopenia and probable sepsis.

## DISCUSSION

Phase II trials in non-small cell lung cancer indicated that both vindesine and cisplatin have activity when used in patients who have not had prior chemotherapy (5–8). When the drugs were combined, a substantial improvement in the CR and PR rate (43%) was noted. In patients receiving high-dose cisplatin (120 mg/m²) and vindesine, the me-

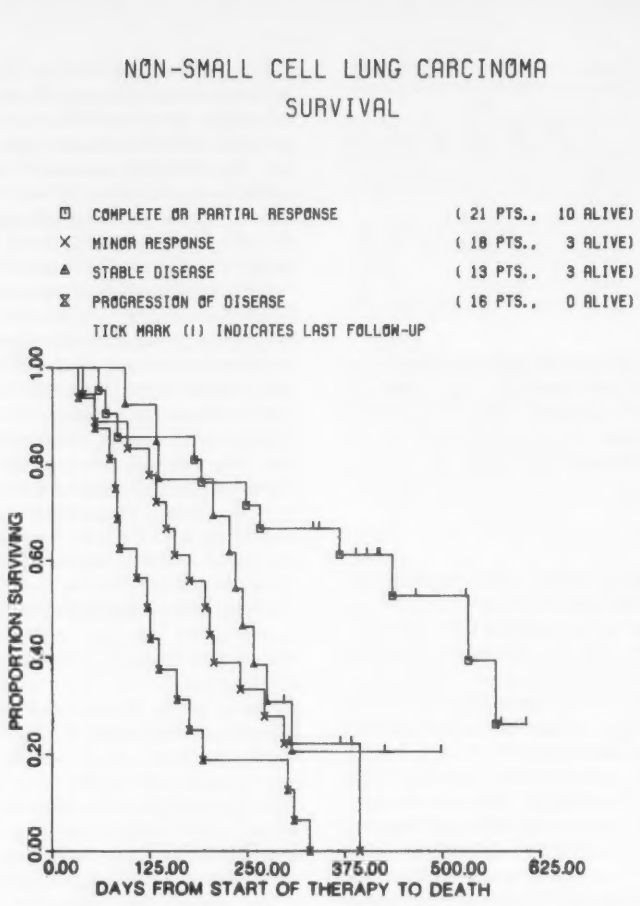


FIGURE 1.—Survival from start of chemotherapy for patients having CR or PR compared to patients having minor regressions, stable disease, or disease progression.

dian durations of response and of survival were significantly longer than in patients receiving low-dose cisplatin (60 mg/m²) and vindesine (response duration was 12 vs 6 months, and survival duration was 22 vs 12 months, respectively). Other phase II trials with vindesine alone have confirmed its activity (14,15), and trials with the two-drug combination are now underway at several centers.

Although it is encouraging, the 43% response rate using these two investigational drugs together still means that > 50% of patients do not achieve a major objective remission. CAP, used by Eagan et al (7,10) in a variety of schedules, is also an active combination, with responses in the 38%–48% range. Therefore, in the current trial, cyclophosphamide and doxorubicin were added to the high-dose cisplatin and vindesine regimen in an attempt to increase the rates and durations of response. High-dose cisplatin (120 mg/m²) was chosen because, as mentioned above, in the earlier randomized study, low-dose cisplatin (60 mg/m²) and vindesine gave an inferior response and survival duration. The doses of cyclophosphamide and doxorubicin were decreased by 25% from those used by Eagan et al in view of the substantially higher cisplatin dose and of the addition of vindesine. Despite this, myelosuppression was moderately severe, with a median wbc count nadir of 2600/mm³; 15% of the patients had severe leukopenia (wbc count < 1000/mm³) on at least one course of therapy. Higher doses of cy-

clophosphamide and doxorubicin would probably have been accompanied by substantially more myelosuppression.

The current study supports the activity seen in earlier trials with both CAP and cisplatin and vindesine. Response rates in all of these trials are similar (31%–48%) (7,9,10,12). The median duration of remission seen with PACE and PCE, however, is more akin to that of cisplatin and vindesine (10 and 12 months, respectively), as are the median survival durations of 18 and 22 months. The toxic effects of the PACE and PCE regimens and the cisplatin and vindesine regimen are also quite similar. Because the response rate is not improved with the addition of doxorubicin and/or cyclophosphamide, we conclude that PACE and PCE has no clear advantage over vindesine and cisplatin. Further disease-oriented phase II trials are needed to identify other active agents for the treatment of non-small cell lung cancer, which can then be added to combinations such as cisplatin and vindesine.

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# 2′-Deoxycoformycin (DCF) and 9-β-D-Arabinofuranosyladenine (Ara-A) in the Treatment of Refractory Acute Myelocytic Leukemia[1,2]

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## SUMMARY

**The combination of 2′-deoxycoformycin (DCF), a potent adenosine deaminase (ADA) inhibitor, and 9-β-D-arabinofuranosyladenine (Ara-A) was used in a patient with acute nonlymphocytic leukemia refractory to all conventional modes of therapy. DCF was given by periodic iv injections to ablate ADA activity. Ara-A was given by continuous iv infusion at an initial dose of 1.5 mg/kg/day, with progressive increases to 6 mg/kg/day. With adequate ADA suppression ($< 2 \times 10^{-2}$ μmols of inosine/hr/$10^6$ cells), the Ara-A decreased the absolute peripheral blood myeloblast count from 36,332 to 780/μl. The patient experienced no renal, hepatic, or neurologic complications during therapy.**

[Cancer Treat Rep 66:253–257, 1982]

Adenosine deaminase (ADA) activity is markedly increased in the peripheral blood of patients with acute leukemia (1). In both in vitro and in vivo studies with 2′-deoxycoformycin (DCF), a potent inhibitor of ADA, the data have suggested that ablation of this enzyme's activity might be selectively cytotoxic to rapidly dividing cells (2–4). In phase I clinical trials, the high doses of DCF required to ablate the ADA activity may have resulted in cell lysis but were often associated with unacceptable toxicity (4). While single-agent, high-dose DCF may be of limited value in the treatment of acute leukemia, its combination with 9-β-D-arabinofuranosyladenine (Ara-A) may be of profound importance.

Ara-A is phosphorylated intracellularly to Ara-ATP, a compound which inhibits DNA polymerase. Although Ara-A has been shown to be a potent antiviral agent in man, its antitumor activity has been limited by its low solubility and by its rapid deamination to 9-β-D-arabinofuranosylhypoxanthine by ADA (5). Therefore, the limited pharmacologic inhibition of ADA by DCF provides a potential mechanism to enhance the antitumor activity of Ara-A. Enhanced cytotoxicity of this synergistic combination and a lack of cross-resistance with cytarabine have been demonstrated both in vitro and in vivo utilizing the L1210 leukemic model in mice (6,7). The feasibility of this combination in man was explored in a patient with refractory acute nonlymphocytic leukemia.

## MATERIALS AND METHODS

### Case Report

A 39-year-old white man was diagnosed as having $M_1$ (FAB classification) acute nonlymphocytic leukemia. Initially, the patient was treated with the standard Southwest Oncology Group induction regimen consisting of doxorubicin at a dose of 40 mg/

[1]Received Feb 23, 1981; revised June 8, 1981; accepted June 23, 1981.

[2]Supported in part by Public Health Service grants CA-26391, CA-09338, and CA-15147 (National Cancer Institute), and HD-12799 (National Institute of Child Health and Human Development) from the National Institutes of Health, Department of Health and Human Services, and by the Veterans Administration Research Service. Dr. Gray is a recipient of an NIH Postdoctoral Fellowship in Oncology, Dr. Grever is a Special Fellow of the Leukemia Society of America, and M. F. E. Siaw is the recipient of a Research Center Development Award (CA-00494).

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$m^2$ by iv push (IVP) on Day 1, 2 mg of vincristine by IVP on Day 1, Ara-C at a dose of 70 mg/$m^2$/day given by 24-hr continuous iv (CIV) infusion on Days 1–7, and prednisone at a dose of 100 mg/day orally on Days 1–5. A complete remission was obtained after two courses of induction therapy. Subsequently, the patient proceeded to consolidation (systemic and CNS) and maintenance therapies.

After two courses of maintenance therapy, the patient experienced a relapse. Reinduction therapy, attempted with two courses of the original induction regimen, did not succeed. One course of AMSA at a dose of 90 mg/$m^2$/day by 24-hr CIV infusion on Days 1–5 was unsuccessful. In the absence of a significant response to therapy, treatment with Ara-A and DCF was initiated. An initial dose of DCF (4 mg/$m^2$) did not adequately suppress ADA activity in the buffy coat. DCF at a dose of 10 mg/$m^2$ by IVP, however, did produce sufficient ADA ablation. After achieving sufficient suppression of ADA activity, the patient started receiving a 24-hr CIV infusion of Ara-A at a dose of 1.5 mg/kg/day. Periodic IVP injections of DCF at a dose of 10 mg/$m^2$ were used to maintain ablation of ADA activity (fig 1).

The Ara-A doses were to be progressively increased (1.5 to 3 to 6 mg/kg/day) until bone marrow hypoplasia or a limiting toxicity became apparent. This drug combination produced an impressive decrease in the absolute number of peripheral blood myeloblasts, however, hypoplastic bone marrow was not achieved at the doses used. Secondary to an extremely prolonged hospitalization and poor prognosis, the patient requested discontinuation of his medical therapy and transfer to a hospital closer to his home. He has subsequently died.

## Source of the Drug

DCF was supplied by the Investigational Drug Branch, National Cancer Institute, Bethesda, MD. Permission to use DCF and Ara-A was obtained from both the Human Subjects Review Committee of the Ohio State University and from the Food and Drug Administration. Informed written consent was obtained from the patient prior to initiation of the study.

## ADA Assay

Sequential determinations of the ADA activity of the peripheral blood buffy coat preparations were obtained by utilizing an assay which measured the conversion of $8\text{-}^{14}C$ adenosine to inosine. The specific details of the enzyme assay were presented in a recent publication (4). One unit of ADA activity is expressed as $10^{-2}$ μmols of inosine formed/hr. Specific activity is expressed as units/$10^6$ cells.

## Serum, Urine, and Cerebrospinal Fluid Nucleoside Determinations

The nucleosides from urine, cerebrospinal fluid, and serum were solubilized using methods previously published, and were analyzed by high-performance liquid chromatography using a Varian 5000 liquid chromatography system (4). Nucleosides in urine samples were resolved in a μBondapak $C_{18}$ column (Waters, Milford, MA). Compounds were eluted isocratically with a mobile phase composed of 10 mM $NH_4H_2PO_4$, pH 4.5, and 5% methanol at a flow rate of 1.5 ml/min. Nucleosides in serum samples were separated on the same column using a mobile phase of 10 mM $NH_4H_2PO_4$, pH 4.5, and 10% methanol at a flow rate of 1.0 ml/min.

## Erythrocyte and Nucleated Cell ATP and dATP Determinations

Ribonucleotides and deoxyribonucleotides were extracted from $1 \times 10^9$ erythrocytes or $1 \times 10^7$ nucleated cells with 1 ml of cold 60% aqueous methanol at –20°C overnight. Following centrifugation, the supernatants were dried and resuspended in water. Recovery from this procedure was 50%–60%. A Partisil-10 SAX ion exchange column (Whatman, Clifton, NJ) was used for quantitation of ATP and dATP from erythrocytes or nucleated cells. Compounds were eluted isocratically using a solvent of 0.45 M $NH_4H_2PO_4$, pH 4.5. Peaks representing adenine nucleotides and deoxynucleotides were identified on the basis of retention times (8). A modification of the DNA polymerase assay was also used to measure the levels of dATP in extracts from both erythrocytes and nucleated cells (4).

Levels of total adenine ribonucleotides and deoxynucleotides in erythrocytes and buffy coat were confirmed by treating the cell extracts (250 μl) with 5 units of alkaline phosphatase (type III, *Escherichia coli*). Protein was precipitated and following centrifugation for 5 mins at $12,000 \times g$, the supernatant was removed and analyzed directly for nucleosides by high-performance liquid chromatography (μBondapak $C_{18}$ column) (4).

## RESULTS

The ADA activity in the peripheral blood was elevated at the start of therapy (24.6 units/$10^6$ cells; fig 1A, Day 1). Normal peripheral blood buffy coat ADA activity has been established as 8.4 ± 4.3 units/$10^6$ cells in our laboratory.

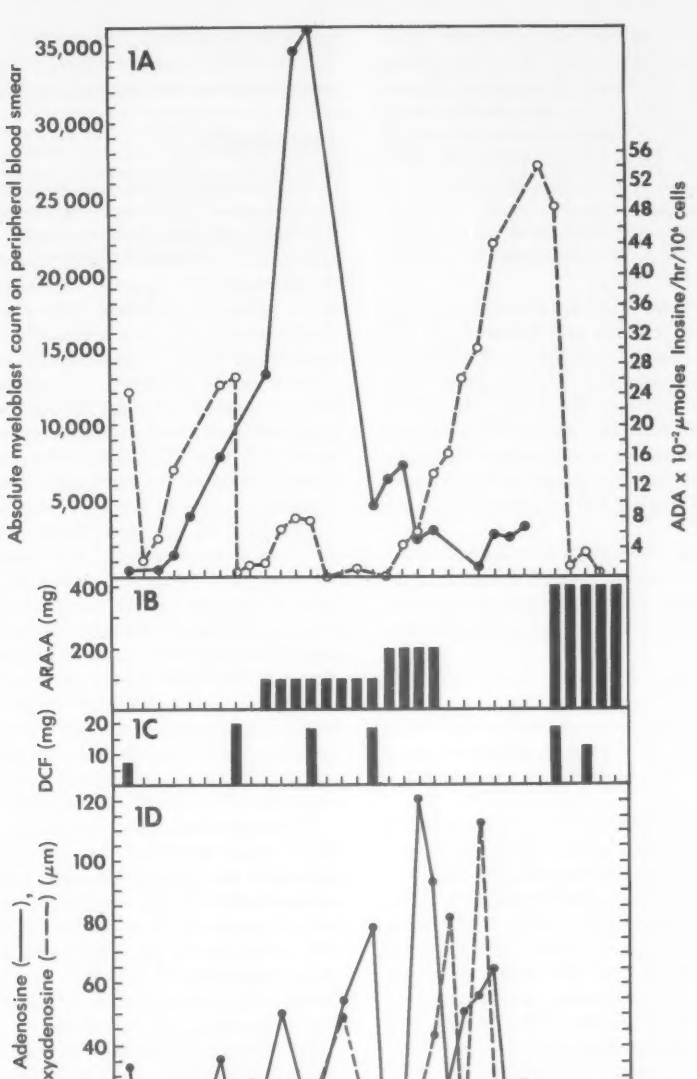


FIGURE 1.—A. Solid line = absolute No. of myeloblasts present in the peripheral blood. Dashed line = specific units of ADA activity ($10^{-2}$ μmols of inosine/hr/$10^6$ cells) in buffy coat preparation. B. Solid bars = total dose of Ara-A given in mg/day. C. Solid bars = total dose of DCF given in mg/day. D. Dashed line = serum deoxyadenosine determinations measured in μM. Solid line = serum adenosine determinations measured in μM.

DCF, as a single agent, had no significant effect on the absolute myeloblast count in the peripheral blood even when the ADA activity in the buffy coat was almost undetectable (fig 1A, Days 8–10). Ara-A was equally ineffective in the presence of detectable ADA activity (fig 1A, Days 10–13). When the buffy coat ADA activity was sufficiently suppressed (< 2 units/$10^6$ cells), the Ara-A infusion produced a profound cytotoxic effect (fig 1A, Days 13–24). The peripheral blood absolute myeloblast count decreased from 36,332 to 780/μl.

An increase in serum adenosine correlated with suppression of ADA activity and followed the decrease in the myeloblast count (fig 1A–D; Days 8–9 and 15–20). With restoration of ADA activity, this nucleoside was cleared from the serum (fig 1A–D; Days 10–11 and 20–22). The increases in serum deoxyadenosine followed the serum adenosine peaks (fig 1D). The maximal serum deoxyadenosine concentration obtained during the study was 16.4 μM on Day 25 (fig 1D).

The cerebrospinal fluid showed minimal accumulation of deoxyadenosine following three injections of DCF. Deoxyadenosine concentrations of 0.6 and 0.7 μM were measured on Day 15 in the cerebrospinal fluid.

The maximal urinary excretion of deoxyadenosine was 4.28 μmols/24 hrs on Day 28. Throughout the period of maximal decrease in peripheral blood myeloblast count, the urinary deoxyadenosine mean concentration was 1.03 μmols/24 hrs (range, 0.18–4.28).

The concentration of dATP did not increase to > 14 pmols/$10^6$ cells in any of the erythrocyte samples which were analyzed. Pretreatment levels of dATP in erythrocytes were < 0.2 pmols/$10^6$ cells. During most of the treatment, dATP levels were 2–7 pmols/$10^6$ cells. Contrary to our earlier results with high doses of DCF, the ATP levels in erythrocytes remained fairly constant and varied between 43 and 82 pmols/$10^6$ cells (4). The dATP levels in buffy coat preparations remained very low. On Day 1 the dATP level was < 5 pmols/$10^6$ cells. By Day 8 the dATP level had increased to 7.8 pmols/$10^6$ cells, but by Days 12 and 13 it had fallen to about 2 pmols/$10^6$ cells. During this time, the ATP levels remained very high in buffy coat cells and varied between 273 and 2392 pmols/$10^6$ cells. The lowest ATP was observed on Day 8, when the dATP was moderately elevated.

The patient's clinical status remained unchanged during the therapeutic trial. The rapid decrease in the peripheral blood myeloblast count produced no renal toxicity. His creatinine was never > 1 mg/dl, and his urine output averaged 4500 ml/day. His SGOT ranged from 28 to 35 units/ml (normal range, up to 40), and his alkaline phosphatase ranged from 89 to 150 units/liter (normal range, 30–94). No neurologic complications were encountered during therapy.

## DISCUSSION

The complete pharmacologic inhibition of ADA by high-dose DCF has been associated with clinical toxicity including CNS dysfunction (lethargy, seizures, and confusion) and reversible liver and renal dysfunction (4). Specific biochemical disturbances were noted to correlate with the clinical toxicity. In those patients, the circulating erythrocytes accumulated high levels of dATP and the ATP was almost totally depleted. Clinical toxicity was manifest at erythrocyte dATP concentrations of ⩾ 40 pmols/$10^6$ cells and at ATP concentrations of < 10 pmols/$10^6$ cells. The CNS abnormalities were temporally related to detectable levels of > 0.9 μM deoxyadenosine in the cerebrospinal fluid.

Contrary to our experience with high doses of DCF as a single agent, the combination of lower doses of DCF with Ara-A in this patient produced none of the previously noted toxicity. Our present patient did not accumulate high levels of dATP in either the erythrocyte or buffy coat cells and ATP was not depleted. The cerebrospinal fluid concentration of deoxyadenosine in this patient did not reach the level that we have previously observed as being toxic. Careful monitoring of these particular biochemical parameters may provide a useful indicator of early clinical toxicity.

The rapid decrement in peripheral blood myeloblast count preceded the increase in both serum adenosine and deoxyadenosine. The increased dATP concentration in the circulating erythrocytes may reflect uptake of these metabolites from the serum with subsequent phosphorylation. The absence of an accumulation of dATP in the circulating myeloblasts may indicate that Ara-A rather than DCF was responsible for cell death.

The principal objective of this study was to determine if pharmacologic inhibition of ADA by DCF would potentiate the effect of Ara-A. This potentiation has been amply demonstrated in animal models, and has a sound biochemical rationale. The combination of these agents and the lack of clinical toxicity should encourage further investigation of this drug combination.

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# Whole-Body Hyperthermia and Chemotherapy for Treatment of Patients With Advanced, Refractory Malignancies [1,2]

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## SUMMARY

**Eleven patients with unresponsive, disseminated cancers received 30 treatments with whole-body hyperthermia (WBH) and chemotherapy. The protocol was designed to treat patients at 42.0°C, 42.2°C, and 42.4°C for 2.5–3 hours (depending on tolerance) in successive treatments given 7–10 days apart. WBH was accomplished in six patients using heated blankets and in five patients by heating extracorporeally circulated blood. Fourteen treatments were at 42.0°C, eight at 42.2°C, six at 42.4°C, and two at 41.8°C. Twenty-seven treatments (90%) lasted ≥ 2.5 hrs. At peak temperature, seven patients received 60 mg/m² of cisplatin iv and four patients received 50 mg/m² of BCNU iv (one patient also received 300 mg/m² of methyl-GAG iv over 2 hrs on each of 3 days prior to WBH and BCNU). Six of ten evaluable patients (60%) achieved a partial remission (median duration, 9 weeks; range, 5–24): two of three patients with melanoma, two of three with colon carcinoma, one of two with sarcoma, and one of two with lymphocytic malignancies. No response advantage was observed for either heating modality or for any drug regimen. Major toxic reactions included hypotension during and after treatment, a "capillary leak" syndrome associated with pulmonary edema, and disseminated intravascular coagulopathy. Two patients died as a result of these complications (both after treatment at 42.0°C). Subjective complaints related to therapy were associated with superficial burns (in patients treated with blankets) and generalized fatigue. Technical advances that have decreased the severe toxicity of treatment are discussed. In addition, methods are proposed to screen for responsive patients and to maximize antitumor interactions of chemotherapy and WBH.**

[Cancer Treat Rep 66:259–265, 1982]

[1]Received Mar 12, 1981; revised July 20, 1981; accepted July 29, 1981.

[2]Supported in part by Veterans Administration General Research Support Funds and by Public Health Service grants CA-23074 and CA-17343 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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Over the last decade, interest in temperature elevation as a treatment for cancer has re-emerged. Temperatures in the range of 42°C–43°C have been shown to amplify the cytotoxic actions of selected chemotherapeutic drugs (1) and radiotherapy (2) as well as being cytotoxic in their own right (3). In addition, it appears that the damaging effects of hyperthermia may be relatively greater in malignant cells than in their normal counterparts (4).

Our interest in whole-body hyperthermia (WBH) began when reports by Pettigrew et al (5), Larkin et al (6), and Bull et al (7) showed the feasibility of this modality and demonstrated that both objective responses as well as palliation could be achieved.

Our own protocol was designed to treat patients at higher temperatures than those used by other investigators, because the degree of hyperthermic cytotoxicity had been shown to depend on the height of the temperature of exposure (3). In addition, we chose a relatively short duration of therapy (2.5–3 hrs at peak temperature) because mammalian cell lines had been shown to develop resistance to thermal killing after 2–3 hrs at temperatures between 42°C and 43°C (8). Subsequently, when our own laboratory investigations (9) indicated that the cytotoxicity due to hyperthermia alone or together with several anticancer drugs was a direct function of the rate of heating, we altered our WBH technique in order to reach cytotoxic temperatures more rapidly. We report here our 2-year experience with this therapy.

## PATIENTS AND METHODS

*Treatment protocol.*—Patients were to receive three treatments with WBH and chemotherapy 7–10 days apart. Depending on tolerance, peak temperatures were to be 42.0°C, 42.2°C, and 42.4°C in successive treatments. When esophageal and rectal probes disagreed, the higher temperature was taken as correct. The treatment durations were to be 2.5–3 hrs, also depending on patient tolerance. At the end of therapy, patients were to be actively cooled to 38°C. Responding patients were eligible for repeat courses after a 3–4-week rest.

*Patients.*—All 11 patients treated had histologic evidence of advanced malignancies refractory to standard treatments. No patient had mass lesions in the central nervous system. Ten patients were male and the median age of the group was 36 years (range, 11–58). The median Karnofsky scale rating (10) was 70% (range, 20%–90%). The tumors treated included three melanomas, three colon carcinomas, three sarcomas, one null cell lymphoblastic lymphoma, and one T-cell acute lymphocytic leukemia. All patients had failed chemotherapy and four had received radiotherapy (*see* table 1).

*Technique of heating.*—All patients were treated under general endotrachial anesthesia using 60% $N_2O_2$ and 40% $O_2$ along with iv barbiturates and narcotics in a standard operating room. The initial six patients were treated essentially by the method of Larkin et al (6). Briefly, after careful padding of pressure points, patients were wrapped in 3-mm thick polyethylene sheets. One water-circulating blanket (Duo-therm pad, American Hospital Supply Corp, McGaw Park, IL) was placed over the patient, one was placed below the patient, and these were then taped together. The blankets were attached to a specially modified K-Thermia Water Circulation Reservoir (model RK-600, Gorman-Rupp Industries, Bellevue, OH), which was capable of heating water to 50°C as well as cooling water to 4°C. During induction of hyperthermia, blanket temperatures were regulated so as to keep skin temperature just below 43°C. Using this technique, the mean time to a core temperature of 42.0°C was 2.8 hrs. The next five patients were treated using the method similar to that described by Parks et al (11). Briefly, an arteriovenous (AV) fistula was made between the femoral artery and vein using a 100-cm silastic shunt tunneled subcutaneously. After 1 week to allow healing, the end of the loop was exposed and cut. The resulting arterial end was connected to a roller-type cardiopulmonary bypass pump (model 5M6002, Travenol Laboratories, Inc, Deerfield, IL). The blood flow was then directed from the pump through a water-to-blood heat exchanger (model 5M0338, Travenol Laboratories, Inc), where the blood was heated to 47°C–48°C. Blood circulation was then directed back to the venous end of the shunt. Blood flow was maintained at 1.2–1.8 liters/minute. Using this technique, the mean time to a core temperature of 42.0°C was 1.3 hrs.

*Patient monitoring.*—Core-temperature monitoring of all patients was accomplished with both a rectal and esophageal resistance thermister (model 702, Yellow-Springs Instruments, Yellow Springs, OH), which had been precalibrated. The resistance was measured by a digital volt meter (model 3400, Precision Corp, Wakefield, MA) allowing conversion into degrees centigrade. All patients had indwelling bladder catheters to monitor urine output. A skin thermister was also attached under the scapula. All patients had continuous ECG monitoring of cardiac rhythm, and selected patients also had placement of a flow-directed, triple-lumen thermodilution pulmonary artery catheter. All patients were treated with an arterial line for blood pressure measurement and access for repeated determinations of blood gasses, cbcs, electrolytes, creatinine, glucose, magnesium, phosphate, calcium, LDH, SGOT, CPK, coagulation profiles, haptoglobin, and serum hemoglobin.

*Fluid replacement.*—When initial patients were treated by the method of Larkin et al (6), fluid replacement was accomplished with 5% dextrose and one-half normal saline alternating with Ringer's lactate at a rate sufficient to maintain urine output at ≥ 20 ml every 15 minutes. This required an average of 700–1000 ml/hr of fluid. When subsequent patients were treated by the method of Parks et al (11), they received 2 units of packed rbcs and 4 units of plasma prior to treatment and were then maintained with about one half as much crystalloid solution (300–500 ml/hr).

*Chemotherapeutic drugs.*—The first of seven patients received 60 mg/m² of cisplatin iv at a rate of 1 mg/minute. The drug was mixed in 500 ml of normal saline with 40 g of mannitol added. The next four patients were treated with 50 mg/m² of BCNU iv by slow push. All chemotherapy was given just as peak temperature was reached. In addition to BCNU, one patient with acute leukemia also received methyl-GAG at a dose of 300 mg/m² iv over 2 hrs on 3 days prior to his second and last treatment. These drugs were chosen because of laboratory data indicating additive cytotoxic interactions between them and hyperthermia (1).[5]

*Criteria of response.*—Complete remission was defined as the complete disappearance of all known disease. Partial remission (PR) was defined as a ≥ 50% reduction in the volume of all measurable disease without the appearance of new lesions. Size of lesions was determined by X-ray studies (including computerized axial tomography), sonography, nuclear medicine scanning procedures, and direct palpation. Duration of response was defined as the time in weeks from satisfaction of the criteria for response until relapse.

[5]Stickney DG, Herman TS, Gerner EW, et al. Inhibitors of polyamine biosynthesis can modulate the development of thermotolerance. Presented at the Third International Symposium on Cancer Therapy by Hyperthermia, Drugs and Radiation, Ft. Collins, CO, June 22–26, 1980.

## RESULTS

Eleven patients underwent a total of 30 WBH and chemotherapy treatments. Six patients had 16 treatments via the blanket method and five patients had 14 treatments via the AV technique. Three patients received four treatments, four patients received three treatments, and two patients received two treatments. Two patients received only one treatment because of early death in one and refusal of the other to be re-treated (due to posttherapy hallucinations) despite a clear PR. Twenty-seven treatments (90%) were for ≥ 2.5 hrs at peak temperatures. In five instances, peak temperature was maintained below the desired temperature for that treatment due to physiologic instability. The peak temperatures reached were 42.4°C in six treatments, 42.2°C in eight treatments, 42.0°C in 14 treatments, and 41.8°C in two treatments.

### Patient Characteristics and Response

Pertinent patient characteristics, method of treatment, temperature attained, and response are shown in table 1. Ten of the 11 patients were evaluable for response. Of these ten patients, six (60%) achieved a PR; there were no complete remissions. Median duration of response was 9 weeks after the criteria for PR were met (range, 5–24 weeks). Three of the six responding patients achieved PR after

TABLE 1.—Patient characteristics and response

| Tumor type | Sex/age (yrs) | Evaluable site | Heating method | Chemotherapy | Peak temperature*(°C) | Response† | Duration of response (wks) | Remarks |
|---|---|---|---|---|---|---|---|---|
| Melanoma‡ | M/48 | Nodes | Blanket | Cisplatin | 42.0 | PR | 6 | Refused further treatment |
| Melanoma | M/51 | Skin | AV shunt | Cisplatin | 42.4 | PR | 12 | |
| Melanoma‡ | M/24 | Skin | AV shunt | BCNU | 42.4 | NR | — | |
| Colon carcinoma | M/58 | Liver | AV shunt | Cisplatin | 42.2 | PR | 5 | |
| Colon carcinoma | M/50 | Liver | AV shunt | Cisplatin | 42.2 | NR | — | |
| Colon carcinoma | M/48 | Liver | Blanket | BCNU | 42.2 | PR | 10 | |
| Ewing's sarcoma‡ | F/36 | Lung | AV shunt | BCNU | 42.4 | PR | 8 | Died after 4th treatment |
| Ewing's sarcoma | M/12 | Lung | Blanket | Cisplatin | 42.0 | NE | — | Died after 1st treatment |
| Fibrosarcoma‡ | M/30 | Lung | Blanket | Cisplatin | 42.2 | NR | — | |
| Lymphoblastic lymphoma | M/25 | Bone marrow | Blanket | Cisplatin | 42.4 | PR | 24 | |
| Acute lymphoblastic leukemia | M/11 | Blood | Blanket | BCNU, methyl-GAG | 41.8 | NR | — | |

*Highest sustained temperature during treatments.
†NR = no response; NE = not evaluable.
‡Previously treated with radiotherapy.

less than three hyperthermia treatments. Two of three patients with melanoma (one with extensive skin lesions and one with nodal disease) achieved a PR. Two of three patients with colon cancer achieved a PR (all patients had hepatomegaly with defects seen on technetium$^{99m}$-labeled sulfur-colloid scan). Of the two evaluable patients with sarcomas (one with Ewing's sarcoma and one with a fibrosarcoma), the patient with Ewing's sarcoma achieved a PR in measured pulmonary lesions apparent on chest films. Of the two patients with lymphocytic malignancies, the patient with T cell acute lymphocytic leukemia in relapse showed a decrease in peripheral blast cells that was considered less than a PR. A 25-year-old patient with lymphoblastic lymphoma (which was diagnosed as null cell), however, had an excellent PR. This patient had massive bone marrow involvement with pancytopenia and circulating blast cells. After his third treatment with WBH (peak temperature, 42.4°C) and cisplatin, his bone marrow was cleared of lymphoblasts and reconstituted normally. Although the patient developed central nervous system involvement, which was treated with radiotherapy and intrathecal chemotherapy, his bone marrow remained without evidence of disease for 24 weeks. In this small series, a determination of which chemotherapeutic regimen was more effective with WBH was not possible.

### Effect of Heating Method

We changed our method of whole-body heating to the more rapid AV shunt technique when laboratory data, which we have reported (9), demonstrated that the cytotoxicity of hyperthermia alone or together with some chemotherapeutic drugs is diminished by slow rates of heating. The shunt technique accomplished WBH to 42.0°C in a mean time of 1.3 hrs as opposed to 2.8 hrs for the blanket method. An improved response rate with faster heating, however, was not apparent within this pilot experience (three patients treated with each technique responded).

## PHYSIOLOGIC RESPONSE AND TOXICITY

### Cardiovascular

Physiologic response and toxicity associated with treatments are presented in table 2.

The mean heart rate of our patients was 166 ± 12 beats/minute at peak temperature, and the cardiac indexes were approximately twice baseline in the four patients (all blanket technique) in whom this measurement was obtained. During therapy, our patients manifested a decrease in mean blood pressure from 105 to 86 (which was similar for both

TABLE 2.—Physiologic response and toxicity

| Parameter | Blanket technique | AV shunt technique |
|---|---|---|
| Cardiovascular* | | |
| Mean heart rate | 164 | 169 |
| Mean blood pressure | 88 | 83 |
| Hematologic† | | |
| No. of treatments causing— | | |
| Neutropenia | 0 | 3 |
| Thrombocytopenia | 2 | 11 |
| Disseminated intravascular coagulopathy | 4 | 6 |
| Neurologic | | |
| No. of patients with— | | |
| Foot drop | 1 | — |
| Seizures | — | 1 |
| Hallucinations | 1 | — |
| Mortality | | |
| No. of patients with— | | |
| Hemorrhage in pulmonary metastasis | 1 | — |
| Hepatic necrosis | — | 1 |

*During WBH treatment.
†Neutropenia = < 1000 cells/mm$^3$; thrombocytopenia = < 70,000 platelets/mm$^3$ (lowest count within 24 hrs of treatment).

techniques), and the decrease was accentuated at the end of therapy when patients were actively cooled. In seven instances mean blood pressure decreased to 60–70 for up to 2.5 hrs after treatment. Toward the end of this study, we found that judicious use of alpha stimulatory sympathomimetic agents (usually 40 μg/ml of phenylephrine hydrochloride as a continuous infusion titrated according to response) avoided precipitous decreases in blood pressure, especially during cooling. Ventricular tachycardia and fibrillation occurred in one patient and ventricular tachycardia occurred in another during therapy, but neither were fatal events. Neither incidence was associated with the use of pressor agents.

Generalized anasarca developed in nearly all patients, and the degree did not appear to be related to method of treatment or temperature attained. This was far more prominent in early patients whose fluid replacement consisted of a high volume of crystalloid solutions as opposed to later patients who were pretreated with colloid solutions and then given smaller amounts of crystalloid solutions during treatment. This apparent "capillary leak" syndrome (pulmonary wedge and central venous pressures were not elevated) was especially hazardous when it occurred in the lungs. In our initial patients, significant increases in alveolar-arterial oxygen gradients were routinely observed by the end of therapy. After changing to colloid preloading, lower volumes of intratreatment crystalloid solutions, and use of positive end expiratory pressure (5–10 cm of $H_2O$) as advocated by Parks et al (11), the occurrence of pulmonary edema was less frequent and less severe.

## Serum Chemistries

Alterations in serum chemistries were similar to those observed by others (6,7).[6] Serum phosphate, magnesium, and calcium generally dropped in the immediate posttreatment period and often required replacement. Serum potassium levels varied without a consistent pattern. Toward the end of this series, iv solutions containing these electrolytes were routinely administered during treatment. The serum enzymes SGOT, CPK, and LDH generally increased during the first 48 hrs following therapy and then decreased to normal in the next 2 days.

[6]Bull JM, Lees DE, Smith R, et al. Whole body hyperthermia combined with nitrosoureas in malignant melanoma. Presented at the 16th Annual Meeting of the American Society of Clinical Oncology, San Diego, CA, May 26–27, 1980.

## Hematologic

Hematologic alterations were common as a result of therapy. While neutrophilic leukocytosis (> 12,000 wbc/mm³) was seen in association with seven treatments in five different patients (all blanket technique), similar to the experience of Larkin et al (6), neutropenia (< 1000 wbcs/mm³) was seen after three treatments (all shunt technique) in three patients whose wbc counts were normal prior to therapy. Despite normal pretreatment levels, platelet counts routinely decreased to < 70,000/mm³ in patients treated with the shunt technique, but a similar decrease in platelet count occurred after only two treatments in two patients treated with the blanket method. Ten treatments (four blanket method and six shunt method) in eight patients were associated with a prolongation of the prothrombin time and partial thromboplastin time and a decrease in the fibrinogen level; replacement of clotting factors was required in six instances. Major bleeding, however, occurred only once (*see* Mortality section). No patients showed evidence of hemolysis.

Severe myelosuppression (neutrophil count < 1000/mm³ and/or platelet count < 50,000/mm³ for ⩾ 5 days following therapy) did not occur in any patient whose counts were normal prior to treatment. In the patients with lymphoblastic lymphoma and acute lymphoblastic leukemia, however, both met the criteria for severe myelosuppression prior to therapy. The patient with lymphoblastic lymphoma showed recovery of marrow production (neutrophils > 2000/mm³ and platelet count > 100,000/mm³) by Day 18 following therapy, but the patient with leukemia did not achieve this level of marrow function.

## Neurologic

Neurologic toxicity was observed in three patients. After three treatments with WBH and cisplatin, one patient developed a bilateral foot drop that improved over 6 weeks (he had received a total of 12 mg of vincristine). Another patient had generalized seizures after therapy and subsequent confusion lasting about 1 week. The third patient reported vivid hallucinations in the intensive care unit following his first treatment. Despite a clear PR, this patient, with widespread melanoma, refused further therapy.

## Cutaneous

Four of the six patients treated with the blanket technique developed second-degree cutaneous burns. These occurred over pressure points such as the heel and buttock areas. One other patient, treated

with the AV shunt technique, also developed a second-degree burn at the point where the venous end of his graft re-entered the subcutaneous space.

### Muscular

Essentially all patients reported severe fatigue, which tended to be more pronounced after the first than subsequent treatments and usually required 3–5 days to improve. Actual muscle weakness was apparent in most patients after therapy, although correlation with CPK elevations was not obvious (electromyographic and nerve conduction studies were not done).

### Mortality

Two patients died as a result of treatment with WBH. The first patient was treated early in our study. He was a 12-year-old boy with Ewing's sarcoma metastatic to his lungs. He had failed all therapy and was bedridden and hypoxic. This patient died 6 hrs after his first treatment at 42.0°C for 2.0 hrs (accomplished with the blanket technique). During the procedure, he had ventricular fibrillation but was resuscitated. Post-mortem examination showed necrosis of multiple pulmonary metastases and hemorrhage into them. Clinically, this patient developed a shock-like syndrome with evidence of disseminated intravascular coagulopathy, pulmonary edema, and severe hypoxia.

The second patient was treated later in the series. She was a 36-year-old female also with Ewing's sarcoma metastatic to her lungs. She had been treated with chemotherapy and radiotherapy, but her tumor nodules were increasing in size. Despite progressive disease, however, her performance status was good. She achieved a PR after three courses of BCNU and WBH via the shunt method. Peak temperatures were 42.0°C, 42.2°C, and 42.4°C in successive treatments. After a 4-week rest she was retreated at 42.0°C for 2.75 hrs. She, too, developed a shock-like syndrome which was also associated with laboratory evidence of severe hepatic injury. At autopsy, necrosis of tumor nodules was observed as well as hepatic necrosis (without tumor involvement of the liver) and moderate cerebral edema. The one obvious difference between the fatal treatment and previous treatments was that a temperature of 42.0°C was achieved in only 25 minutes as compared with 60, 78, and 56 minutes in prior treatments.

## DISCUSSION

In this trial, six of ten evaluable patients achieved a PR after treatment with WBH and chemotherapy, confirming the reports of others (5,11,12).[6] In addition, we found that higher peak temperatures than other investigators have used (5–7,11,13) could be tolerated by patients. Theoretically, these higher temperatures should have resulted in a greater cell kill (3) and an increase in response rate, although this was not observed in our limited series.

Difficulty in administering therapy and of caring for patients whose physiology was deranged by treatment were obstacles to our use of this technique. We eventually found, however, that judicious use of alpha stimulatory sympathomimetic drugs, pretreatment fluid loading with colloid solutions, use of smaller amounts of crystalloid replacement during treatment, and use of positive end expiratory ventilation as advocated by Parks et al (11) decreased the magnitude of the capillary leak syndrome and consequent pulmonary edema, which were major sources of risk to patients.

Despite meticulous care, however, two of our 11 patients died as a result of treatment with WBH. It is noteworthy that neither death was associated with treatment at the higher peak temperatures since both patients died after treatment at 42.0°C, a core temperature routinely employed by others (6,11,13). Our 12-year-old patient's cardiopulmonary status was not able to withstand the stress of WBH. He was treated early in our series but would not have been considered for treatment later when it became obvious that rigid performance criteria are mandatory. In addition, he was treated before we had improved our technique as outlined above.

The reason for the second patient's death is less clear. This 36-year-old female was treated late in the series and had previously received three WBH treatments via the AV shunt technique, without undue toxicity (the third was at 42.4°C for 2.5 hrs). The fourth treatment was given at 42.0°C for 2.75 hrs. The one significant difference from previous treatments was that a peak temperature of 42.0°C was reached more quickly. In only two other instances was a core temperature at 42.0°C attained in $< 30$ minutes, although no increased toxicity was noted for these treatments. We made an effort to reach cytotoxic temperatures rapidly when our laboratory data (9) indicated that the cytotoxicity of heat, alone or together with some chemotherapeutic agents, is a direct function of the speed of heating. At autopsy this patient showed predominantly hepatic necrosis (without tumor involvement of the liver). It is likely that the toxic effects of hyperthermia and chemotherapeutic drugs on normal as well as malignant tissues are increased by rapid heating. In addition, rapid heating may accentuate temperature differences between organ sites, resulting in relative hot spots. The AV shunt method

makes rapid heating possible, but lower peak temperatures may then prove to be the limit of tolerance, and our decision to treat at 42.0°C may not have represented a sufficient decrease. Others who treat with WBH should be cognizant of the potential increase in toxicity as a result of more rapid heating.

The utility of any treatment must be measured by toxicity versus benefit. WBH in our experience and in that of others (5,11,12)[6] can cause regression of tumor masses in a significant percent of heavily pretreated patients, but median duration of remissions tends to be short (only 9 weeks in the present series). We continue to be interested in this technique, however, because occasional patients, like our patient with lymphoblastic lymphoma, have excellent responses. In an effort to screen patients for response to hyperthermic therapy, our laboratory, in conjunction with Dr. David Alberts, has employed the human tumor cloning procedure developed by Salmon et al (14). We have grown a total of 26 tumors and have seen a wide range of sensitivity to hyperthermia alone or together with anticancer agents in vitro. Unfortunately, clinical correlation has not yet been possible, but there is the hope that this technique may be able to delineate patients who will have significant responses to thermochemotherapy.

It also remains encouraging that human malignancies commonly respond to WBH and chemotherapy, despite the fact that the concurrent use of these modalities is probably not optimum. There is a lack of knowledge concerning the metabolism, proper dose, and scheduling of anticancer drugs at hyperthermic temperatures. Indeed, although chemotherapeutic agents are generally administered after peak temperatures are achieved (11,13)[6] [because of in vitro observations indicating maximum synergism with this scheduling (1)], recent microvasculature studies (15) have shown that hyperthermia may increase blood flow into normal tissues significantly more than into tumors. Diminished drug delivery into malignant tissues would, therefore, be expected when chemotherapeutic agents are given after hyperthermic temperatures are reached and would suggest that prior administration is preferable. Further, it may well be that our original presumptions (ie, that treatments at high temperatures over relatively short time intervals would optimize anticancer effects) are incorrect. Read et al[7] have shown that the thermotolerance that develops in cells after 4–6 hrs at 41°C lasts only 10–20 hrs, after which cell killing again proceeds exponentially. Perhaps very long duration treatments at temperatures nearer 41°C would provide a greater therapeutic index.

If the technique of delivering WBH advances (we are now using only the shunt method), if screening of patients for responsiveness proves feasible, and if better understanding of the use of chemotherapeutic drugs with WBH is gained, this application of thermochemotherapy may play a significant part in the future treatment of patients with cancer. Because our current knowledge is rudimentary, however, it is our opinion that WBH must remain a research procedure and should not be offered as "standard" therapy at this time.

[7]Read RA, Fox MH, Bedford JS, et al. Thermotolerance and chronic hyperthermia. Presented at the Third International Symposium on Cancer Therapy by Hyperthermia, Drugs and Radiation. Ft. Collins, CO, June 22–26, 1980.

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# Treatment of the Blast Crisis of Chronic Myelogenous Leukemia With 5-Azacitidine and VP-16-213[1,2,3]

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## SUMMARY

**Twenty-seven patients with chronic myelogenous leukemia in blast crisis were treated with a combination of 5-azacitidine (150 mg/m²/day iv in 3 divided doses × 5 days) and VP-16-213 (75 mg/m²/day × 5). No patient had lymphoid histology, and terminal transferase was not present in the 12 patients tested. One complete and 15 partial responses (58% response rate) were seen, with a median survival of 231 days for responders and 73 days for patients who failed to achieve a partial response ($P = 0.008$). The overall survival was 161 days (range, 20–316+). Moderately severe nausea and vomiting, muscle aches, and mucositis (two patients) were the major side effects, although toxic effects tended to decrease with subsequent courses and many patients could be treated on an outpatient basis. These results compare favorably with other results in this refractory disorder and suggest that 5-azacitidine and VP-16-213 be studied further, either in higher doses or in combination with other agents, in therapy for chronic myelogenous leukemia in blast crisis.**

[Cancer Treat Rep 66:267–271, 1982]

Despite effective therapy for the chronic phase of chronic myelogenous leukemia (CML), most patients will develop either an accelerated myeloproliferative phase refractory to treatment or blastic leukemia indistinguishable from acute leukemia. A variety of agents with activity in acute myelogenous leukemia have been ineffective in substantially prolonging survival in patients with either the accelerated or blast crisis stages of CML. Although reports suggest that patients in blast crisis with lymphoblastic morphology (1), hypodiploid cytogenetics (2), and/or terminal transferase (TdT) activity (3) may have an improved response rate following treatment with vincristine and prednisone, the overall survival for this group of patients is still < 20% at 1 year.

5-Azacitidine is a pyrimidine nucleotide analog which is active in acute myelogenous leukemia but which has not been tested adequately in blast crisis of CML. In a recent review of the activity of 5-azacitidine, hematologic improvement was noted in three of nine CML patients (4). In other, unpublished observations, partial responses were noted in three patients treated with 5-azacitidine at the National Cancer Institute. These responses were transient, however, and thus, although it appears that 5-azacitidine has some activity in blast crisis, it would probably not be very useful as a single agent.

In this study, therefore, 5-azacitidine was combined with VP-16-213, a semisynthetic podophyllotoxin derivative, which has an action similar to vincristine in that it produces an arrest in the cell cycle in metaphase, producing a marked reduction

[1]Received Apr 1, 1981; revised July 9, 1981; accepted July 20, 1981.

[2]Sponsored by the NCI-Pan American Health Organization Collaborative Cancer Treatment Research Program.

[3]Presented in part at the annual meeting of the American Association for Cancer Research, San Diego, CA, 1980, and at the International Society of Hematology/International Society of Blood Transfusion, Montreal, Canada, 1980.

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[6]We thank Pat Duffey, RN, for her assistance in this project.

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in the mitotic index. VP-16-213 has been inadequately tested in CML, although a partial response was noted in a patient treated orally with VP-16-213, and a complete response has been described following treatment of blast crisis with another podophyllotoxin, VM-26 (5,6). A study completed at the Baltimore Cancer Research Center in patients with advanced acute nonlymphocytic leukemia demonstrated that combination therapy with 5-azacitidine and VP-16-213 was tolerable, although no complete responses were noted in this group of previously treated patients with advanced disease (7). In the present report, we describe results of the treatment of 27 patients with CML in blast crisis, which indicate that the combination of 5-azacitidine and VP-16-213 is active in this disease and warrants further study.

## PATIENTS AND METHODS

### Patient Eligibility

Patients were considered to have blast crisis of CML if they had > 50% blasts and progranulocytes in the bone marrow on two successive occasions and/or > 30% peripheral blood blasts and progranulocytes on two successive occasions at least 1 week apart, unless an obvious rapid rise in count supervened. They were eligible for this study if they had had no chemotherapy for 2 weeks preceding the experimental therapy and no prior exposure to 5-azacitidine or VP-16-213 and if they had no splenic irradiation or corticosteroid therapy during this trial. Patients presenting initially with acute leukemia with the presence of a $Ph^1$ chromosome were eligible for treatment in this study.

### Chemotherapy

5-Azacitidine (150 mg/m²) was administered by iv push every 8 hours for 5 days. The drug was administered through a free-flowing iv setup within 30 minutes of reconstitution. VP-16-213 (75 mg/m²/day) was given by iv infusion over 1 hour for 5 days. Repeat administration of the chemotherapy during induction was determined by evaluation of the bone marrow and peripheral blood on Day 14 of therapy. If the peripheral blast count was consistently ≥ 5000/mm³, then treatment was repeated on Day 14 without testing additional bone marrow, but if the peripheral blast count was < 5000/mm³, a bone marrow aspirate and/or biopsy was done on Day 14. If the marrow was 0–1+ cellular, therapy was withheld and a repeat marrow test was done 1 week later. If the marrow was 2+ cellular and contained > 40% blasts, then retreatment was begun. A maximum of three courses of induction therapy were administered. Doses of 5-azacitidine were increased by 50 mg/m²/day in subsequent courses if nonhematopoietic toxicity from a previous course of therapy was tolerable, while doses of VP-16-213 were increased by 25 mg/m²/day. Patients whose disease progressed after two courses of therapy were considered treatment failures and were removed from the study.

All patients who achieved complete or partial remission received maintenance therapy with the same drugs every 8 weeks. 5-Azacitidine was given at a dose of 100 mg/m² iv × 5 days in three daily divided doses, and VP-16-213 was given at a dose of 50 mg/m²/day iv for 5 days. All patients received allopurinol, prophylactic platelet transfusion, and empiric antibiotic therapy for infection.

Complete response was defined by the following criteria: (*a*) bone marrow containing < 5% blasts, with normal cellularity or granulocytic hyperplasia consistent with the chronic phase of CML; (*b*) peripheral blood counts that had returned to a wbc count < 12,000/mm³, with < 5% myelocytes and a sustained platelet count > 150,000/mm³; (*c*) elimination of all signs of extramedullary disease noted prior to therapy; and (*d*) a spleen that was no longer palpable. Partial response was defined as reduction of the percent of blasts in the marrow to < 30%, accompanied by normal peripheral blood counts.

Patient survival was calculated from the initiation of therapy.

## RESULTS

All of the patients treated were evaluable for response and toxic effects. Characteristics of the patient population are summarized in table 1. No patient had received prior therapy for the blast crisis phase of the disease. It should be noted that TdT was absent in the 12 patients treated in Baltimore whose blasts were tested. None of the 27 patients had lymphoid histology, as determined by morphologic criteria and cytochemical staining. Most of the patients had massive splenomegaly. Five of the patients presented initially in blast crisis ($Ph^1$-positive acute leukemia). The median duration of the chronic phase was 3 years (range, 6 months to 10 years) in the other 22 patients. All had been treated with busulfan, and some had also received hydroxyurea during the chronic phase.

One complete and 15 partial responses were seen, for an overall response rate of 58% (table 2). The complete response was achieved in a patient initially presenting in blast crisis and was of 120 days' duration; relapse occurred after two courses of maintenance therapy. Two nonresponders died with

TABLE 1.—Patient characteristics*

| Characteristic | |
|---|---|
| No. of patients | 27 |
| Sex | |
| Males | 20 |
| Females | 7 |
| Age (yrs) | |
| Median | 43 |
| Range | 19–65 |
| Ph[1] chromosome | 26 |
| TdT | 0† |
| Myeloid histology | 27 |
| Splenomegaly (> 6 cm) | 23‡ |
| Initial blast count (cells/mm$^3$) | |
| Median | 24,000 |
| Range | 7000–140,000 |
| Presented in blast crisis | 5 |

*Unless otherwise indicated, values = No. of patients.
†12 patients were tested.
‡1 of the 27 patients had splenectomy.

TABLE 2.—Response to treatment*

| Response | No. of patients |
|---|---|
| Complete | 1 |
| Partial | 15 |
| No response | 11† |

*Marrow aplasia occurred in 4 of the 27 patients.
†5 patients had transient increases in platelet and granulocyte counts; 2 patients died with aplasia.

aplastic bone marrows and five had transient platelet and granulocyte count increases following therapy. Although most marrows became hypoplastic after treatment, marrow aplasia occurred in only four of the 27 patients. Responders received a median of three courses of therapy (range, two to eight), while nonresponders received a median of two courses (range, one to four). Dose escalation was possible for the first two or three courses in most patients.

The overall survival curve and the survival curves for responders and nonresponders are shown in figure 1. Seven patients are still alive, three of whom

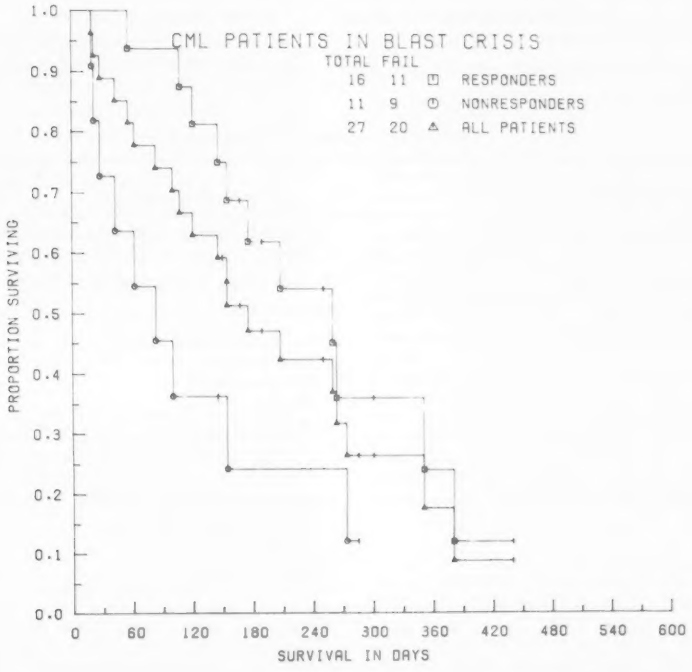


FIGURE 1.—Survival of responders, nonresponders, and total patient population, plotted from start of treatment.

are still in the study, receiving maintenance therapy. The median survival for the entire group of patients was 161 days (range, 20–316+). Patients who responded had a median survival of 231 days (range, 56–316+), with a median survival of 73 days (range, 20–273) for nonresponders ($P = 0.008$). Patients initially presenting in blast crisis had a median survival of 175 days (range, 154–310+).

The toxicity of these agents was similar to that described previously and was largely attributable to the 5-azacitidine. Seventeen patients had significant daily nausea and vomiting, although in many patients, this tended to decrease with subsequent courses. Although maintenance therapy was discontinued in two patients because of these gastrointestinal problems, most other patients could in fact be treated primarily as outpatients. Eleven of 27 patients developed muscular aches and weakness, which were severe in two patients. Hypokalemia (potassium < 3 mEq/liter) developed in five patients but was not clearly correlated with muscle weakness. Mucositis developed in five patients but was clinically severe in only two; in one of these patients, bleeding from the hypopharynx, with subsequent pulmonary aspiration, was the immediate cause of death. Infectious complications requiring antibiotic therapy developed in about two thirds of the patients and approximately one half of the patients required platelet transfusion. Significant and persistent abnormalities in hepatic and renal function occurred in only one patient, in whom massive tumor lysis occurred during the first course of therapy; severe hyperphosphatemia, hypocalcemia, azotemia, and hyperbilirubinemia occurred and had begun to improve prior to his death from pharyngeal hemorrhage. Creatinine elevations attributable to chemotherapy were not seen in any other patient, but there was no rigorous testing for the more subtle abnormalities of proximal tubular function that have recently been described with 5-azacitidine used in combination with a variety of other drugs (8).

## DISCUSSION

This study demonstrates that the combination of 5-azacitidine and VP-16-213 has significant activity in the treatment of patients with CML in blast crisis. The patient population treated was representative of the spectrum of patients seen with this disorder and consisted of consecutive patients treated at each institution. The incidence of positive tests for TdT was somewhat lower than that in other reports in the literature (3), but in our experience in the Baltimore Cancer Research Center, only about 10% of the patients in blast crisis are TdT-positive.

These results compare favorably with other reports of chemotherapy in this notoriously refractory disorder. The results of a number of other studies

TABLE 3.—Comparison with other studies

| Investigator (ref No.) | No. of patients | Therapy/histology* | Median survival (days) |
|---|---|---|---|
| Coleman et al (11) | 202 | HU, 6-MP, PRED, VCR ± DNR | 84 |
| Vallejos et al (12) | 39 | COAP, DOAP + single agent | 110 |
| Spiers et al (13) | 19 | TRAMPCOL | 203† |
| Rosenthal et al (1) | | VCR, PRED, ARA-C, 6-TG | |
| | 73 | Total patients | 120 |
| | 46 | Lymphoblastoid | 180 |
| | 21 | Myeloid | 90 |
| Janossy et al (14) | | VCR/PRED base | |
| | 26 | ALL− | 70 |
| | 11 | ALL+ | 250 |
| Johnson and Goldman (10) | 24 | Intensive chemotherapy + TBI, with autologous stem cell reconstitution | 139 |
| Present study | 27 | 5-AZA, VP-16-213 | 161 |

*HU = hydroxyurea; 6-MP = 6-mercaptopurine; PRED = prednisone; VCR = vincristine; DNR = daunorubicin; COAP = cyclophosphamide, VCR, cytarabine (ARA-C), and PRED; DOAP = DNR, VCR, ARA-C, and PRED; TRAMPCOL = 6-thioguanine (6-TG), DNR, ARA-C, methotrexate, PRED, cyclophosphamide, VCR, and L-asparaginase; TBI = total body irradiation; 5-AZA = 5-azacitidine. ALL−, ALL+: defined by presence or absence of blast TdT, morphology, and reactivity with antibody against lymphoid leukemia cells (ref 14).

†8 of the 19 patients had "good responses," with median survival of 203 days in the responders.

are summarized in table 3, and it can be seen that the overall survival in our study was superior to that in other studies reported for myeloid histology and was in the same range as survival reported for lymphoid histology. These results are also similar to the overall results of superintensive therapy followed by marrow transplant rescue using autologous stem cells obtained and cryopreserved during the chronic phase of the disease (9). Although initial reports (9) using this novel approach were promising, a recent update indicates that early relapses occur commonly, with a median overall survival of 139 days in 24 patients (10). It should also be noted that therapy with 5-azacitidine and VP-16-213 was reasonably well-tolerated and that, after the first course of therapy, most patients spent most of their time out of the hospital. The maintenance therapy was not unduly myelosuppressive, and relatively few patients required either platelet transfusions or hospitalization for complications during maintenance. Although the survival of responding patients remains < 1 year, responders had a significant prolongation of survival, indicating a benefit from therapy and the desirability of further studies of therapy in this disorder. Improved survival in responding patients has also been noted in other studies (2,11–14).

These responses were seen even though marrow aplasia occurred in only a few patients, suggesting that improved results might be seen with more intensive therapy. A preliminary report of a study using this combination to treat acute nonlymphocytic leukemia in children has noted improved results with higher doses of both drugs (15). Because of this, our followup study utilizes higher doses, with the 5-azacitidine being given as a continuous infusion in an attempt to ameliorate some of the gastrointestinal side effects (16,17).

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# Phase II Study of Procarbazine in Small Cell Carcinoma of the Lung [1,2]

**Anders Gersel Pedersen,[3] Sverre Sörenson,[4] Kristian Aabo,[3] Per Dombernowsky,[5] and Heine H. Hansen[3,*]**

## SUMMARY

**Thirty-nine patients with small cell anaplastic carcinoma of the lung were given oral doses of procarbazine ranging from 100 to 200 mg/m² on Days 1–14 every 4 weeks. All patients had previously received combination chemotherapy. No responses were observed in the 24 evaluable patients. In reviewing the literature, we were unable to find evidence that the inclusion of procarbazine in combination chemotherapy is beneficial in small cell anaplastic carcinoma. Accordingly, it is concluded that procarbazine should not be considered as a useful drug in the management of this cell type of lung cancer.**

[Cancer Treat Rep 66:273–275, 1982]

One of the major problems in the management of small cell carcinoma of the lung (SCCL) is to maintain an induced remission; this is presumably due to a selection and overgrowth of resistant cells during treatment (1). Cytotoxic agents with efficacy against these cells are therefore needed. In the 1960s, trials focusing on the use of procarbazine (Natulanar) in SCCL (2–6) led to its extensive use in combination regimens (7,8). These early phase II trials, however, do not satisfy contemporary standards with respect to number of patients, uniformity of histology, criteria of response, performance status, and stage of disease.

To elucidate this uncertainty, we undertook a phase II trial of procarbazine.

## PATIENTS AND METHODS

Eligibility criteria included histologically confirmed SCCL, measurable or evaluable disease as evidenced by physical examination or chest X-ray, no prior treatment with procarbazine, wbc count $\geq 4000/mm^3$, and platelet count $\geq 100,000/mm^3$, unless bone marrow involvement was documented. A 2-week rest from previous therapy with other cytostatic compounds (6 weeks for the nitrosoureas) or radiation was required.

Physical examination, chest X-ray, and measurement of serum electrolytes, creatinine, alkaline phosphatase, LDH, SGOT, bilirubin, and prothrombin time were performed at entry in the study and every 4 weeks thereafter. Cbcs were performed every 2 weeks.

The drug regimen used was procarbazine (100 mg/m²/day) given orally on Days 1–14 of each 4-week cycle. Dose modifications were made according to wbc and platelet counts. If wbc counts were between 2000 and 3000/mm³ and/or platelet counts were between 75,000 and 100,000/mm³, the dose was reduced to 67%. If wbc counts were $< 2000/mm^3$ and/or platelet counts were $< 75,000/mm^3$, the treatment was postponed. If wbc counts were $> 4000/mm^3$ and platelet counts were $> 100,000/mm^3$, the dose was escalated by 25%.

Because of minimal hematologic toxicity observed at a dose of 100 mg/m²/day, the initial dose was subsequently escalated to 150 mg/m² and finally to 200 mg/m². Treatment was continued until objective evidence of disease progression or unacceptable toxicity occurred. Patients were considered evaluable if they had received at least one treatment cycle.

[1]Received Dec 15, 1980; revised July 10, 1981; accepted Aug 21, 1981.

[2]Supported by a grant from Esper and Olga Boel's Foundation.

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TABLE 1.—Patient characteristics

| Characteristic* | Evaluable | Total |
|---|---|---|
| No. of patients | 24 | 39 |
| Age (yrs) | | |
| Median | 58 | 60 |
| Range | 36–75 | 36–75 |
| Sex | | |
| Males | 17 | 29 |
| Females | 7 | 10 |
| Prior therapy | | |
| Chemotherapy only | 22 | 35 |
| Chemotherapy + radiotherapy | 2 | 4 |
| Karnofsky performance status (%) | | |
| Median | 75 | 65 |
| Range | 50–90 | 40–90 |
| Stage of disease | | |
| Regional | 9 | 15 |
| Extensive | 15 | 24 |

*Unless otherwise specified, values = No. of patients.

Evaluation of response and toxicity was done according to World Health Organization criteria (9).

## RESULTS

There were 39 patients included in the trial. Pretreatment characteristics are summarized in table 1. All patients had received prior therapy, including at least three of the following drugs: cyclophosphamide, methotrexate, VP-16-213, doxorubicin, vindesine, and vincristine. The doses used were: 100 mg/m² (16 patients), 150 mg/m² (16), and 200 mg/m² (seven). Eight patients were unevaluable because of early death due to progressive disease; 31 were evaluable for toxicity, but only 24 were evaluable for response, because seven patients discontinued therapy within the first 4 weeks due to gastrointestinal toxicity (table 2). No responses were observed. Median duration of treatment was 44 days (range, 28–95).

## DISCUSSION

The results of procarbazine therapy in this trial, which included patients with SCCL refractory to standard treatment, were disappointing. The treatment had to be discontinued in a high percentage of cases, due to nausea and vomiting, despite the use of antiemetics; antitumor activity was not observed. Earlier phase II trials focusing on the efficacy of procarbazine have been performed on previously untreated patients and, with the exception of the trial by the European Organization for Research on Treatment of Cancer (4), have included few patients with the same histologic type of lung cancer. It is, therefore, not surprising that a wide range of response frequencies (8%–85%) have been observed (2–6). It is conceivable that the group of patients treated in this study were too heavily pretreated for this phase II trial to be conclusive. However, in equally pretreated patients with the same performance status, we have found drugs such as VP-16-213 and vindesine (10,11) to be active. Furthermore, at present it is of prime interest to find drugs active in otherwise refractile clones and not drugs with a spectrum of activity identical to that of drugs already in use. In most combination regimens, the dose of procarbazine has been 100 mg/m²/day on Days 1–14 every 4 weeks (7,8,12–15).

However, Samuels et al (6) suggest that a dose of 200 mg/m² is necessary when a drug is used as a single agent in carcinomas.

In our study, no responses were seen on any of the three dose levels, including 200 mg/m².

Several groups have reported activity of combination chemotherapy including procarbazine in patients already resistant to cyclophosphamide, methotrexate, and CCNU (12,14,16). Since these combinations include compounds known to be active against SCCL, it is impossible to assess the importance of procarbazine. In another study including 15 patients, Abeloff et al (15) used cyclic alternating

TABLE 2.—Response and toxicity

| Dose (mg/m²/day) | No. of patients | No. of responses/ No. of evaluable patients | Toxicity grade* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Gastrointestinal | | | Hematologic | | |
| | | | 4 | 3 | 0 | 1 | 2 | 3 |
| 100 | 16 | 0/12 | 4 | 3 | 12 | 2 | 1 | 0 |
| 150 | 16 | 0/9 | 6 | 3 | 5 | 1 | 4 | 0 |
| 200 | 7 | 0/3 | 5 | 1 | 2 | 1 | 1 | 2 |
| Total | 39 | 0/24†,‡ | 15/31‡ | 8/31 | 19/31 | 4/31 | 6/31 | 2/31 |

*Ref 9; values = No. of patients.
†8 were excluded because of early death.
‡7 discontinued therapy within the first 4 wks.

combinations consisting of cyclophosphamide, doxorubicin, and VP-16-213, followed on Day 56 by vincristine, methotrexate, BCNU, and procarbazine. Only one additional response was observed after initiation of the second combination. Eagan et al (8) compared cyclophosphamide plus vincristine followed by either procarbazine or methotrexate and found a deleterious effect of procarbazine since five of seven patients had disease progression with procarbazine after an initial response to cyclophosphamide and vincristine. Likewise, in 40 patients, Vogelzang et al (17) found no beneficial effect when procarbazine and vincristine were added to CCNU and doxorubicin.

Alexander et al (7) combined procarbazine, CCNU, vincristine, and cyclophosphamide in an attempt to give two drugs with supposed intracerebral activity. In comparing the effects of these compounds with those of cyclophosphamide, vincristine, and methotrexate, they were unable to detect any difference in the frequency of intracerebral metastases in 23 patients with SCCL.

In conclusion, we have not been able to demonstrate that procarbazine has any activity as a single agent in patients with small cell carcinoma resistant to known active compounds. Furthermore, we have not been able to obtain evidence from the literature indicating that the inclusion of procarbazine in combination therapy has a beneficial effect.

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# Phase I Study of High-Dose Mitomycin With Autologous Bone Marrow Support [1,2]

**Gregory P. Sarna,* Richard Champlin, John Wells, and Robert Peter Gale [3,4]**

Recently, intensive multidrug or multimodality cancer therapy, followed by the infusion of previously cryopreserved autologous bone marrow to protect against marrow aplasia, has been studied in patients with advanced malignancy refractory to standard therapy (1). Occasional responses have been reported, but substantial morbidity and mortality have been associated with this approach. In these high-dose multiagent studies, the relative contribution of each agent to efficacy and toxicity frequently was not clear.

We have performed a phase I study of mitomycin given at high doses and followed by the infusion of previously cryopreserved autologous bone marrow. Our primary goal in this study was to assess the nonhematologic toxicity of this regimen. Secondary goals were to screen for tumor response and to assess the pharmacokinetics of high-dose mitomycin. Long-range goals of this and planned similar studies of other agents would be to establish a data base which would facilitate the optimization of intensive multiagent therapy combined with autologous bone marrow transplantation. Mitomycin was selected for this study because it has a relatively broad spectrum of clinical activity and because, in conventional doses, myelosuppression is the primary dose-limiting toxicity (2).

## MATERIALS AND METHODS

Patients were considered eligible for this study if they met the following criteria: advanced, pathologically defined malignancy refractory to or lacking standard therapy; ambulatory status with an expected survival ≥ 12 weeks; age ≤ 60 years; bone marrow that was free of malignancy and had cellularity ≥ 30%; normal hematologic status (wbc count ≥ 4000/mm$^3$, granulocyte count ≥ 1500/mm$^3$, and platelet count ≥ 150,000/mm$^3$); normal hepatic status (SGOT ≤ 50 mg/100 ml, bilirubin ≤ 1.3 mg/100 ml, and prothrombin time ≤ 15 seconds); normal renal status (creatinine ≤ 1.4 mg/100 ml); no therapy during the 4 weeks preceding marrow storage or mitomycin therapy; and no prior pelvic irradiation.

Eligible patients participating in this study signed an informed consent that was approved by the UCLA Human Subject Protection Committee. Mitomycin was administered under "Investigational Exemption for a New Drug" 16,735 issued by the US Food and Drug Administration.

Patients, under general anesthesia, had bone marrow harvested from bilateral posterior iliac crest regions. Details of subsequent bone marrow preparation, fractionation, cryopreservation, and thawing have been reported (3,4). In brief, a Ficoll-Hypaque separation was performed on the bone marrow suspension diluted with Hanks' balanced salt solution by layering Ficoll-Hypaque under the bone marrow suspension and by centrifuging this material in an RC-3 centrifuge (DuPont Instruments, Newtown, CT) at 1450 rpm for 30 minutes. The mononuclear cell band was collected, washed, and resuspended in McCoy's 5A medium with 10% fetal bovine serum at a concentration of $10^7$ to $3 \times 10^7$ cells/ml. DMSO was added at 10% with the cells on ice. The cells were frozen in 5-ml vials, using a Cryo-Med freezing system (Mt. Clemens, MI). For infusion, the marrow was thawed rapidly at 37°C, then slowly diluted threefold at room temperature with McCoy's 5A medium with 1% bovine serum. This was pumped into a bag for administration to the patient. Using this procedure, we have found that a fractionated yield of $10^7$ to $2 \times 10^7$ nucleated

[1]Received Mar 9, 1981; revised June 30, 1981; accepted July 14, 1981.

[2]Supported in part by Public Health Service grants CA-15688 and CA-23175 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Gale is a scholar of the Leukemia Society of America.

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[4]We thank Ms. S. McCarthy, our house staff, and our nurses for assistance in the care and evaluation of these patients and Drs. Donald R. Van Harken and M. Dianne De Furia of Bristol Laboratories for their assistance with mitomycin assays.

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marrow cells/kg or of $5 \times 10^4$ myeloid stem cells (CFU-GM)/kg is sufficient for engraftment. In our laboratory, the normal range of CFU-GM colonies/$10^5$ marrow cells plated is 119 ± 53 for unfractionated marrow and 560 ± 177 for fractionated marrow (90.6% ± 34.5% recovery). Aliquots of thawed bone marrow were assayed at the time of infusion for nucleated cell content, for viability by trypan blue exclusion, and for myeloid stem cell content (5).

Patients received high-dose mitomycin either shortly after bone marrow cryopreservation or after the failure of intervening therapy. Mitomycin was administered by rapid (30-minute) iv infusion once daily for 3 days. Five patients received doses of 20 mg/m²/day × 3; one patient received 40 mg/m²/day × 3. Thawed bone marrow was infused iv 48 hours after the last dose of mitomycin.

In four patients, sera were obtained at predetermined intervals following the Day-1 and Day-3 doses. These were assayed for mitomycin concentrations by an *Escherichia coli* inhibition assay performed through the courtesy of Bristol Laboratories (6). Twenty-four-hour urinary excretion of mitomycin was also assessed.

Patients received intensive medical support in standard isolation rooms. Toxicity was assessed with physical examination, repetitive blood tests, and other studies as indicated.

Response to therapy was defined as *complete response*—the disappearance of all measurable or evaluable lesions for ≥ 4 weeks; *partial response (PR)*—a ≥ 50% decrease in the sum of the products of the diameters of measurable lesions, lasting ≥ 4 weeks and without the appearance of new lesions; *minimal response*—an objective shrinkage of disease that does not meet the criteria of a PR but persists ≥ 4 weeks without the appearance of new lesions; or *disease progression*—a ≥ 50% increase in the sum of the product of diameters of measurable lesions, the appearance of new lesions, or tumor-induced death. All other responses were considered to be stable.

Patient characteristics and mitomycin doses are shown in table 1. All patients except one had received previous chemotherapy. Three patients (Nos. 1–3) received mitomycin starting 1 day after marrow procurement and cryopreservation. Three patients (Nos. 4–6) received mitomycin after intervening therapy 16, 6, and 4 weeks, respectively, after marrow procurement and cryopreservation.

## RESULTS

### Patient Entry

This study was closed after six patient entries. Patients were scheduled to enter the study in groups

TABLE 1.—Patient characteristics

| Mitomycin dose (mg/m²/day) | Patient No.* | Age (yrs)/sex | Malignancy | Previous therapy† |
|---|---|---|---|---|
| 20 | 1 | 47/female | Pancreatic adenocarcinoma | None |
| 20 | 2 | 41/male | Lung adenocarcinoma, locally advanced | Radiation, MTX, Vcr, CTX, Adr, CCNU |
| 20 | 3 | 56/male | Colon adenocarcinoma metastatic to lung, liver, and bone | 5-FU, Vcr, CCNU, radiation |
| 40 | 4 | 38/male | Colon adenocarcinoma metastatic to liver and lymph nodes | 5-FU, MeCCNU |
| 20 | 5 | 33/male | Diffuse histiocytic lymphoma involving lymph nodes, soft tissue, and pleura | CTX, Adr, Vcr, Pred, MTX/CF, L-Asp, Bleo, radiation |
| 20 | 6 | 22/male | Teratocarcinoma and embryonal cell carcinoma of the testis metastatic to lung and liver | Vbl, Bleo, Plat, Adr, VP-16-213, Hex, Mith |

*Patient Nos. correspond to UCLA bone marrow transplantation patient Nos. as follows: 1 = 9020; 2 = 9022; 3 = 9023; 4 = 9024; 5 = 9027; 6 = 9028.

†Adr = doxorubicin; Bleo = bleomycin; CTX = cyclophosphamide; L-Asp = L-asparaginase; MeCCNU = methyl-CCNU; Mith = mithramycin; MTX = methotrexate; MTX/CF = high-dose MTX with citrovorum factor rescue; Plat = cisplatin; Pred = prednisone; Vcr = vincristine; Vbl = vinblastine.

of three at each tolerable dose. After three entries at a dose of 20 mg/m² × 3, patient 4 was entered at a dose of 40 mg/m² × 3. Because that dose was associated with unacceptable nonhematologic toxicity (*see* below), entry was resumed at the original dose of 20 mg/m² × 3 until the study was closed due to further unacceptable nonhematologic toxicity.

## Marrow Infusion

Infused marrow contained an average of $1.9 \times 10^9$ nucleated cells ($2.67 \times 10^7$/kg; range, $0.46 \times 10^9$ to $2.9 \times 10^9$, or $0.96 \times 10^7$/kg to $4.2 \times 10^7$/kg). The mean viability of nucleated cells was 77% (range, 52%–100%), and the mean number of viable nucleated cells infused was $1.53 \times 10^9$ ($2.10 \times 10^7$/kg; range, $0.24 \times 10^9$ to $2.76 \times 10^9$, or $0.5 \times 10^7$/kg to $2.7 \times 10^7$/kg). The mean CFU-GM content infused was $7.9 \times 10^6$ cells ($1.2 \times 10^5$/kg; range, $3.2 \times 10^6$ to $16 \times 10^6$, or $0.36 \times 10^5$/kg to $2.67 \times 10^5$/kg).

## Toxicity

All patients demonstrated substantial early hematologic toxic effects, except patient 6, who died on Day 9. Hematologic parameters tended to drop precipitously, with leukopenia and neutropenia occurring within 8–10 days and thrombocytopenia occurring somewhat later. Recovery of hematologic elements generally occurred by 3–4 weeks. Patients were subject to substantial risk of infection or bleeding for 1½–3 weeks. Hematologic toxicity and recovery data are shown in table 2. There was no clear relationship between time to hematologic recovery and the number of viable nucleated cells or CFU-GM infused. All patients with leukopenia developed fever. Two patients had *Pseudomonas aeruginosa* bacteremia. No bleeding episodes due to thrombocytopenia occurred.

Nonhematologic toxic effects are shown in table 3. The five patients treated at a dose of 20 mg/m²/day × 3 had mild to moderate nausea and vomiting. Alopecia occurred in all patients surviving ≥ 2 weeks. One patient developed superficial phlebitis in the venous system draining an injection site in the forearm. Stomatitis was severe in two patients. Immediately after transplant, patient 5 developed hepatomegaly, right upper quadrant pain, and ascites. These toxic effects were resolved over 3–4 weeks. He died of progressive lymphoma 7 weeks after mitomycin therapy. Post-mortem examination revealed diffuse hepatic veno-occlusive disease. Fibrinous pleuritis and pericarditis, perhaps related to therapy, were also noted at autopsy.

Diarrhea was mild to moderate in three patients and severe in two. In one of the two patients, profound enterocolitis was the immediate cause of death. This patient also had hemorrhagic pancreatitis and possible early hepatotoxicity.

The patient who received a dose of 40 mg/m²/day × 3 also had an early death due to profound enterocolitis associated with sepsis, bleeding, hemorrhagic pancreatitis, and agonal azotemia. Other renal, pulmonary, and cardiac toxic effects were not seen. Only one patient, however, was at risk for such toxic effects beyond 12 weeks.

## Tumor Response

Two patients (Nos. 3 and 5) had brief PRs followed by rapid tumor progression and death. One additional patient (No. 1) had a minimal response associated with subjective improvement and survival of about 1 year. One patient (No. 2) had disease progression despite therapy. Two patients (Nos. 4 and 6) were not evaluable for response because of early death. Response and survival data are presented in table 3.

TABLE 2.—Hematologic mitomycin toxicity

| Patient No. | Dose (mg/m²/day) | Wbc count (cells/mm³) 1st day* ≤ 500 | Wbc count (cells/mm³) 1st day* > 1000 | Wbc count (cells/mm³) No. of days ≤ 500 | Neutrophil count (cells/mm³) 1st day* < 100 | Neutrophil count (cells/mm³) 1st day* > 500 | Neutrophil count (cells/mm³) No. of days ≤ 100 | Platelet count (cells/mm³) 1st day* < 20,000 | Platelet count (cells/mm³) 1st day* > 20,000 unsupported | Platelet count (cells/mm³) No. of days < 20,000 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 8 | 22 | 14 | 10 | 20 | 9 | 14 | 20 | 6 |
| 2 | 20 | 10 | 30 | 20 | 10 | 30 | 19 | 12 | 28 | 16 |
| 3 | 20 | 10 | 28 | 17 | 10 | 28 | 15 | 18 | 28 | 10 |
| 4† | 40 | 6 | | | | | | 6 | | |
| 5 | 20 | 8 | 24 | 14 | 8 | 26 | 12 | 12 | 28 | 16 |
| 6‡ | 20 | | | | | | | | | |

*First day of therapy in which blood cell counts were at these levels. Day 1 was the first day of mitomycin therapy; bone marrow was infused on Day 5.

†Patient 4 died on Day 6; neutrophil count was 480/mm³ on Day 6.

‡Patient 6 died on Day 9; on Day 8, wbc and neutrophil counts were 3900/mm³ and platelet count was 80,000/mm³.

TABLE 3.—Nonhematologic mitomycin toxicity and patient course

| Patient No. | Mitomycin dose ($mg/m^2$/day) | Toxic effects | Response | Survival |
|---|---|---|---|---|
| 1 | 20 | Nausea, vomiting, mild diarrhea, alopecia, and mild transient elevation of alkaline phosphatase and SGOT at 5 wks | Minimal | 55 wks |
| 2 | 20 | Nausea, vomiting, severe stomatitis and diarrhea, alopecia, and mild elevation of alkaline phosphatase at 3 wks | Disease progression | 10 wks |
| 3 | 20 | Nausea, vomiting, severe stomatitis and diarrhea, alopecia, and mild elevation of alkaline phosphatase and bilirubin at 2 wks | PR | 12 wks |
| 4 | 40 | Fatal bowel toxicity, hemorrhagic pancreatitis, and azotemia with shock | Not evaluable | 6 days |
| 5 | 20 | Hepatic toxicity (veno-occlusive) with ascites, mild diarrhea, alopecia, and fibrinous pleuropericarditis | PR | 7 wks |
| 6 | 20 | Fatal bowel toxicity, hemorrhagic pancreatitis, and hepatotoxicity | Not evaluable | 9 days |

## Mitomycin Pharmacokinetics

Mitomycin disappeared rapidly from the serum and was not detectable 6 hours after injection. Figure 1 shows the mean and range of serum drug concentrations as a function of time, at a dose of 20 $mg/m^2$/day. Drug concentrations on Days 1 and 3 were similar at this dose level for each patient and were pooled in this figure. The estimated $\alpha$-half-life is 10 minutes and the estimated $\beta$-half-life is 86 minutes. In the patient treated with the 40-$mg/m^2$ dose, drug concentrations were measured on Days 1 and 3. They were found to be 2.0 and 9.9 $\mu$g/ml immediately after injection, 1.7 and 2.0 $\mu$g/ml at 5 minutes, 0.5 $\mu$g/ml at 1 hour, and undetectable at 6 hours. Urinary excretion of mitomycin over 24 hours averaged 9% (range, 3%–19%).

## DISCUSSION

At the conventional dose of 20 $mg/m^2$, mitomycin toxicity is usually limited to immediate nausea and vomiting and delayed and cumulative myelosuppression. Mucositis and diarrhea may also occur. Other toxic effects including alopecia, cellulitis, skin rash, pulmonary fibrosis, nephrotoxicity, and possibly cardiotoxicity and pleuropericarditis are rare but have been reported (2,7). Myelosuppression, however, is generally regarded as the dose-limiting toxicity of mitomycin.

Mitomycin has been used primarily in the treatment of adenocarcinoma of the stomach or pancreas. It is also active in a wide range of other tumor types including breast cancer, non-small cell carcinoma of the lung, and colorectal carcinoma. The broad spectrum of clinical activity of mitomycin and its relatively moderate nonhematologic toxic effects at a standard dose led us to undertake this phase I trial of high-dose mitomycin followed by autologous bone marrow infusion in patients with resistant cancer.

Hematologic toxicity, as expected, was severe and led to febrile and infectious complications. Unlike a standard dose of mitomycin, which causes delayed marrow suppression and wbc and platelet count nadirs at 3½ to 4 weeks, high doses cause abrupt and early myelosuppression. Based on the pattern of bone marrow recovery from standard doses, one might have expected endogenous marrow recovery to be delayed 5–6 weeks. The more rapid recovery time observed in this study (3–4 weeks) suggests that transfused marrow may have hastened recovery. Such an inference, however, is speculative since no patients were studied without marrow infusion. Hematologic toxicity was least in patient 1, who had not had previous chemotherapy.

High doses of mitomycin also produced nonhematologic toxicity. At the dose of 20 $mg/m^2$/day × 3, fatal toxic effects occurred in one of five patients. This toxicity manifested as severe enterocolitis with mucosal sloughing, bloody diarrhea, and hemorrhagic pancreatitis. Similar toxic effects also occurred in the patient who received 40 $mg/m^2$/day × 3. Lesser degrees of bowel toxic effects occurred in other patients at the 20-$mg/m^2$/day dose.

The substantial bowel toxicity associated with high-

dose mitomycin is not prominent when the drug is administered in conventional doses. Studies in dogs given high-dose mitomycin with bone marrow support, however, have revealed prominent bowel toxicity when the drug was given as a single dose at 40 or 60 mg/m² (8). A preliminary report of other studies of high-dose mitomycin in man did not reveal bowel toxic effects in 11 patients treated with doses of mitomycin ranging from 30 to 50 mg/m² (9). Nephrotoxicity, a major toxicity in preclinical studies,

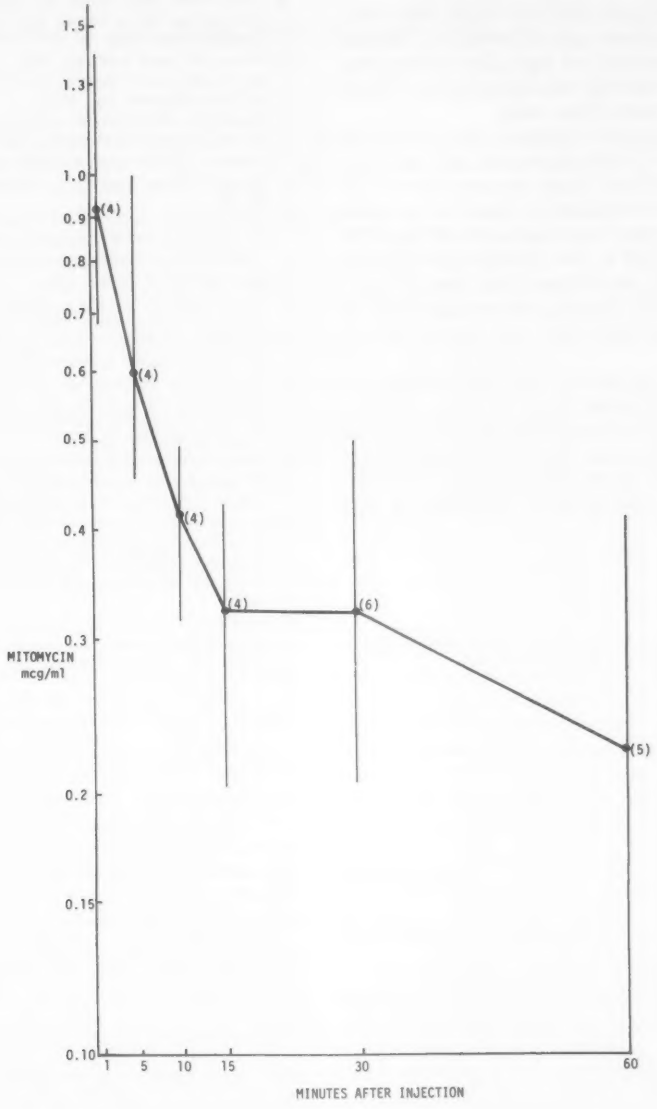


FIGURE 1.—Mean plasma mitomycin concentration as a function of time after the iv injection of 20 mg/m². Range of values and No. of observations are expressed for each data point.

was not seen in this study. Only one patient, however, was at risk beyond 90 days.

Hepatic veno-occlusive disease has been previously reported with other intensive chemotherapy regimens (10,11) and occurred in at least one of our patients. This has been seen in other studies of high-dose mitomycin (12).

One minimal response and two brief PRs were seen in the four patients who survived > 2 weeks. Considering the toxicity of high-dose mitomycin, the level of clinical activity was felt to be insufficient to justify continuation of the study.

The pharmacokinetics of the drug given at the 20-mg/m$^2$ dose level to these patients was not substantially different from those reported in the literature, albeit with considerable variation between patients and with a somewhat longer β-half-life (2,13). In the patient studied at the 40-mg/m$^2$ dose level, there was no significant change other than a higher initial peak level. The Day-3 peak concentration of 9.9 μg/ml seems unduly high and cannot be explained.

We have studied, therefore, high-dose mitomycin with subsequent infusion of previously cryopreserved autologous bone marrow in six patients with resistant tumors. Although this regimen had clinical activity, substantial bowel and pancreatic toxicity and some hepatic toxicity limit the utility of this approach.

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# Clinical and Pharmacologic Evaluation of Split-Dose Intermittent Therapy With Dianhydrogalactitol [1,2]

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## SUMMARY

**Dianhydrogalactitol was administered as two 1-hr infusions separated by a 4-hr period once every 5 weeks to 21 patients with advanced solid tumors. Total doses ranged from 100 mg/m² (50 mg/m² twice on a single day) to 160 mg/m² (80 mg/m² twice on a single day). Peak concentrations of drug at the end of a 1-hr infusion ranged from 1.9 to 5.6 μg/ml. Plasma elimination of dianhydrogalactitol was approximated best by a two-compartment open model. The α-half-life was 3.9 ± 1.9 mins and the β-half-life was 31.3 ± 2.7 mins. Dose-limiting hematologic toxicity was encountered at a total dose of 160 mg/m², with leukopenia occurring more frequently than thrombocytopenia. Other toxic effects included mild to moderate nausea in most patients and two instances of moderate alopecia. One patient with large cell cancer of the lung had a partial regression lasting 2 months and one patient with a carcinoid in the thymus had a partial regression lasting 7½ months. A third patient with a mixed adenocarcinoma-squamous cell carcinoma of the lung had improvement (30% decrease) in his pulmonary tumor for > 2 months.**

[Cancer Treat Rep 66:283–287, 1982]

In 1976, we reported our phase I study results of a 5-day intermittent schedule of dianhydrogalactitol (DAG) in 50 adult patients with solid tumors (1). Based on that study, phase II–III studies of DAG using a dose of 25–30 mg/m²/day iv × 5 days every 5 weeks were conducted in patients with cancers of the lung (2), colon (3), breast (4), and brain (5) and in those with melanoma (6). Some antitumor activity was noted for DAG in squamous cell and large cell lung cancers and in gliomas. In 1977, Barranco and Flournoy (7) reported on the cell killing, growth kinetics, and recovery of dividing and nondividing CHO cells exposed to DAG in vitro. They determined that nondividing cells were almost twice as sensitive to DAG as dividing cells. They noted that nondividing and dividing cells were more sensitive to DAG when it was administered in two dose fractions 4–8 hrs apart than when the same total integral dose was given as a single exposure. Based on those data, we elected to conduct a clinical and pharmacologic evaluation of a split-dose intermittent schedule for DAG in man.

## METHODS

### Patients and Treatment

To be eligible for this study, patients had to have (*a*) a histologic diagnosis of cancer that was unlikely to be responsive to any established mode of therapy, (*b*) normal wbc and platelet counts, (*c*) no prior chemotherapy with nitrosoureas, and (*d*) no prior chemotherapy within 3 weeks. They also had to have signed an informed consent document. During the interval between treatments, wbc counts were obtained twice weekly and platelet counts were obtained once weekly.

[1]Received Mar 10, 1981; revised Aug 24, 1981; accepted Sept 10, 1981.

[2]Supported in part by SPF-8200 from the Department of Oncology, Mayo Foundation, and by the Helen C. Levitt Fund, Mayo Foundation.

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Twenty adults and one teenager with advanced solid tumors were treated with DAG (supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD). Eleven patients had lung cancer (four squamous cell, four large cell, one adenocarcinoma, one squamous cell and adenocarcinoma, and one carcinosarcoma), two had sarcoma (synovial cell and osteogenic), one each had adenocarcinoma of the pancreas, colon, rectum, cervix, and unknown primary, and one each had melanoma, mesothelioma, and carcinoid of the thymus. The median age was 56 years (range, 16–73) and the mean Eastern Cooperative Oncology Group (ECOG) performance status (PS) was 1.8 (range, 1–3). Fifteen patients had had prior chemotherapy (only three with a single agent) and 13 had had prior radiation therapy.

We chose a starting dose of 100 mg/m$^2$, which is two thirds of the total maximum tolerable dose of 150 mg/m$^2$ when DAG is given on a 5-day schedule by rapid iv push. DAG was given as two 1-hr infusions separated by 4 hrs. The drug was dissolved in 125 ml of 5% dextrose in water. The starting dose was 50 mg/m$^2$ × 2 (total dose, 100 mg/m$^2$), with escalations to 60, 66, 72.5, and 80 mg/m$^2$ × 2. The interval between treatment was 5 weeks. The highest total cumulative dose (787 mg/m$^2$) was given in six courses at 5–6-week intervals to a patient with squamous cell lung cancer. Drug doses were escalated for individual patients if toxicity permitted.

### Pharmacologic Evaluation

Pharmacologic studies were done in eight patients, each receiving two 1-hr infusions of DAG, 4 hrs apart, at doses of 60–72.5 mg/m$^2$. Four milliliters of blood was taken at 45 and 60 mins during the infusions and at intervals up to 3 hrs following the completion of each infusion. Urine samples were collected from one patient after each infusion and at various times for 24 hrs after the first infusion.

The assay for DAG in plasma and urine was carried out exactly as described by Munger et al (8) except for modification of the mobile phase used for chromatography. Plasma or urine (1 ml) was added to a 16 × 125-mm screw cap plastic culture tube followed by 1.0 ml of distilled water, 0.5 ml of phosphate buffer (0.1 M, pH 7.0), and 0.5 ml of a freshly prepared aqueous solution of 5% diethyldithiocarbonic acid (Sigma Chemical Co, St. Louis, MO). The tube was capped, vortexed, kept at room temperature for 1 hr, and frozen (–10°C) until analyzed. We confirmed the finding of Munger et al (8) that derivatization of DAG in plasma at concentrations up to 50 μg/ml was complete under these conditions.

Samples were thawed and transferred with a 0.5-ml distilled water wash to 16 × 125-mm teflon-lined screw cap glass culture tubes, and 8.0 ml of chloroform was added to each. The tubes were shaken on a mechanical shaker for 10 mins and centrifuged (1500 × *g*, 15 mins). The aqueous layer was removed by aspiration, leaving the material at the interface and the lower layer behind. A 33% NaCl solution (4 ml) was added and the sample was vortexed to disperse the solid materials. Following centrifugation and aspiration, the NaCl wash was repeated. Seven milliliters of the chloroform extract was transferred to a 15-ml conical centrifuge tube and evaporated to dryness under a stream of nitrogen. Chloroform (1 ml) was added to wash the walls of the tube and evaporated under a stream of nitrogen. The sample was then dissolved in 200 μl of chloroform and 10 μl was injected for analysis. The derivative was extracted qualitatively as reported by Munger et al (8).

High-pressure liquid chromatography was carried out with a Laboratory Data Control System employing a variable wavelength detector at 254 nm. A Whatman PXS-1025 PAC column (4.6 × 250 mm) at ambient temperature was used for the analysis employing a mobile solvent system of heptane/ethanol/glacial acetic acid (60/40/0.02). The flow was 2 ml/min and the pressure was approximately 700 pounds per square inch. The lower limit of detection was approximately 30 ng/ml of plasma. Linear standard curves were obtained with concentrations from 100 ng/ml to 50 μg/ml of plasma ($r^2$ = 0.99). Data were subjected to pharmacokinetic analysis using the nonlinear least-squares regression program NONLIN (9).

## RESULTS

### Pharmacologic Studies

The plasma elimination of DAG is shown in figure 1. Peak concentrations of the drug at the end of the infusions ranged from 1.9 to 5.6 μg/ml (mean, 2.7), with no correlation to the dose administered between 60 and 72.5 mg/m$^2$. The disposition of DAG was approximated best by a two-compartment open model. There were no significant differences (paired t test) between the rate constants for the first or second infusions. The mean ± SE values (n = 16), assuming elimination from compartment 1, were $k_{12}$ (0.161 ± 0.04 min$^{-1}$), $k_{21}$ (0.33 ± 0.15 min$^{-1}$), and $k_{10}$ (0.3 ± 0.01 min$^{-1}$). The corresponding half-lives calculated from the disposition rate constants were 3.9 ± 1.9 mins for the rapid initial α-half-life and 31.3 ± 2.7 mins for the biologic β-half-life. These calculations do not include data from one patient whose computer estimates of α- and β-half-lives were

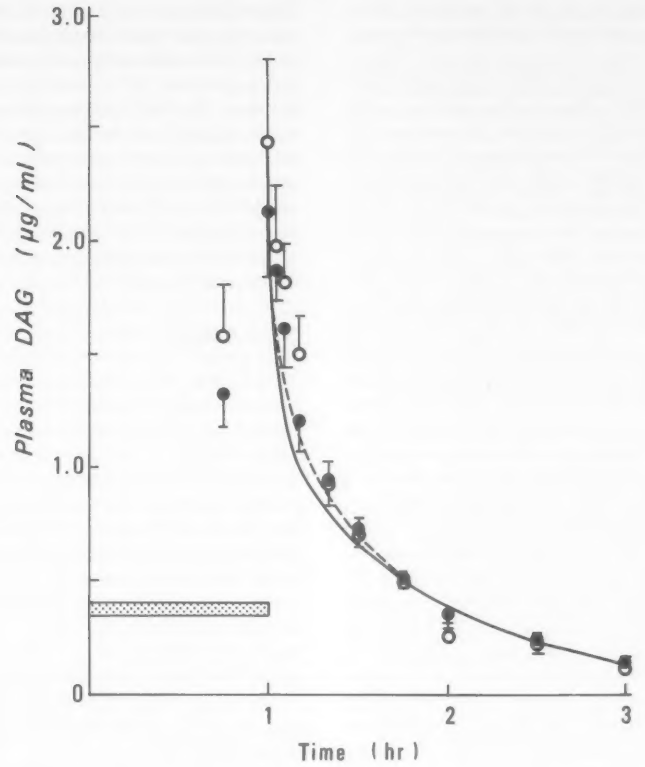


FIGURE 1.—Plasma concentrations of DAG during and following 60-min infusions (solid bar) in 8 patients at doses from 60 to 72.5 mg/m². Each patient received 2 infusions, 1 in the morning (○) and 1 4 hrs later (●). Bars = SEM. Curves are computer simulations of rates of plasma elimination following the 1st infusion (solid line) and the 2nd infusion (broken line).

considerably longer than the other seven patients, although peak plasma levels and final levels (at 3 hrs) were comparable to the group. This patient had normal serum creatinine and liver function tests.

One patient had abnormal liver function with a 50% increase in SGOT and another patient had slightly decreased renal function with a 20% increase in serum creatinine prior to treatment. Disposition of the drug in these patients was similar to that in patients with normal liver and renal functions. Urinary excretion of unchanged drug was measured in one patient. Only 4.4% of the total dose was excreted as intact drug in 24 hrs.

## Toxicity

Qualitatively, the toxicity of the single-day split-dose DAG was almost identical to the toxicity of the daily × 5-day schedule for DAG, with the exception that two cases of moderate alopecia developed on the split-dose schedule and no alopecia was observed on the 5-day schedule. Nausea and vomiting of a mild to moderate nature was observed in most of the patients and was more severe than that seen with the 5-day schedule.

Thirty-eight courses of chemotherapy were evaluable for hematologic toxic effects among the 21 patients: 21 courses at doses of 100–120 mg/m² and 17 courses at doses of 132–160 mg/m². Twelve of the 21 lower dose courses (57%) were associated with neutropenia, with a median wbc count nadir of 3000/mm³ (range, 1800–3900) on median Day 16 (range, 10–26). Very mild thrombocytopenia (lowest platelet count nadir, 97,000/mm³) occurred in only four of 21 lower dose courses (19%) on median Day 13 (range, 11–18). At the higher dose levels,

leukopenia was noted in six of 17 courses (35%), with a median wbc count nadir of 1800/mm³ (range, 700–3200) on median Day 15 (range, 10–18). Five of 17 (29%) of the higher dose courses were associated with thrombocytopenia, with a median platelet count nadir of 98,000/mm³ (range, 18,000–118,000) on median Day 14 (range, 13–17). In summary, leukopenia occurred more frequently than did thrombocytopenia, but both were comparable in severity. Of the six patients treated with first courses of DAG at the dose levels of 145 and 160 mg/m² (three each), five of six developed leukopenia (range, 700–3200 cells/mm³) and three of six developed thrombocytopenia (range, 18,000–112,000 cells/mm³). Since only two of six had had prior single-agent chemotherapy (5-FU) and three of six had had prior radiation therapy, we felt that escalation of the dose to a level > 160 mg/m² was not warranted. We believe the higher incidence of leukopenia in the patients treated at doses < 160 mg/m² was due to extensive prior combination chemotherapy and radiation therapy. The most severe hematologic toxicity (wbc count nadir 700/mm³, platelet count nadir 18,000/mm³) occurred after administration of 160 mg/m² to a 71-year-old female (ECOG PS of 2) with a metastatic adenocarcinoma in the liver from an unknown primary site. Her serum creatinine was 1.9 mg/dl and her alkaline phosphatase and SGOT were twice normal. Bilirubin was normal. Also at a dose of 160 mg/m², a 38-year-old male (ECOG PS of 2) with a carcinosarcoma of the lung developed leukopenia only (wbc count nadir, 900/mm³). He had no liver metastases and normal liver function tests and serum creatinine. Another patient with liver metastasis, a total serum bilirubin of 1.1 mg/dl (direct, 0.8 mg/dl), an alkaline phosphatase six times normal, and an SGOT 3½ times normal had mild leukopenia only (wbc count nadir, 3200/mm³) at a dose of 145 mg/m². The only patient with ascites (adenocarcinoma of the cervix) had normal liver and kidney function, and no hematologic depression at a dose of 132 mg/m².

### Responses

Twenty of the 21 patients had measurable or evaluable tumors. Three tumor responses were noted. One patient with a mixed adenocarcinoma-squamous cell carcinoma of the lung had an improvement (30% reduction) in the pulmonary tumor for 2+ months. This patient had had no prior chemotherapy. The DAG dose was 145 mg/m². Another patient had a regression of his large cell cancer of the lung for 2 months after a starting dose of 132 mg/m². This patient previously had had partial regression following initial chemotherapy with the combination of cyclophosphamide, doxorubicin, and cisplatin. He relapsed and failed to respond to maytansine prior to DAG administration. The third patient had a partial regression of 7½ months in his carcinoid of the thymus. He had failed combination chemotherapy with cyclophosphamide, doxorubicin, DTIC, and vincristine; with bleomycin, doxorubicin, and cisplatin; with cyclophosphamide, methotrexate, and prednisone; and single-agent therapy with maytansine. He had a 2-month partial regression with the combination of dactinomycin and vinblastine prior to treatment with DAG.

## DISCUSSION

Our data demonstrate that in man DAG is removed rapidly from plasma with almost no drug remaining 3 hrs after termination of a 1-hr infusion. The average α- and β-half-lives of DAG in our patients (3.9 and 31.3 mins, respectively) are similar to the values of 2.8 and 26.6 mins reported in the dog by Kimura et al (10) using a gas chromatographic assay specific for DAG. A β-half-life of 43.7 mins has been reported in the rat using a high-pressure liquid chromatographic assay (11). A much longer secondary half-life of 123 mins was found in man by Eckhardt et al (12) for the disappearance of [1-³H]-DAG, followed by a third slower phase of elimination with a half-life of up to 24 hrs. Ten percent to 30% of the radioactivity was attributed to metabolites (5).

In in vitro studies of CHO cells, Barranco and Flournoy (7) found that a concentration × time value for DAG of 4.2 μg/ml/hr was required to reduce the survival of exponentially growing cells by 63%, and 2.4 μg/ml/hr was needed to reduce the survival of nondividing cells by 63%. Exponential decreases in cell survival were not achieved until dividing or nondividing cells were exposed to concentrations > 12.5 μg/ml/hr. Dividing and nondividing cells were more sensitive to DAG given in two dose fractions 4–8 hrs apart than to the same integral dose given in a single exposure. In the present study, peak concentrations of DAG were > 2 μg/ml and concentrations exceeded 1 μg/ml for only 50 mins, from 30 mins after start of the infusion to 20 mins after termination of the infusion. It is apparent that concentrations of DAG per time of exposure achieved in our patients, even assuming intercellular concentrations equal to those in plasma, were considerably lower than those required for a 63% kill of CHO cells in vitro. We do not know if human tumor cells have sensitivities to DAG comparable to CHO cells. The fact that three patients with histologically diverse tumors (mixed adenosquamous cell carcinoma of the lung, large cell carcinoma of the lung, and

carcinoid of the thymus) had tumor shrinkage suggests that some human tumors may be quite sensitive to DAG, at least more sensitive than CHO cells in vitro.

The low rate of tumor response in this group of patients at doses of DAG producing maximal acceptable toxicity does not warrant further study of the schedule involving two 1-hr infusions. However, we believe it is of interest to determine if the antitumor activity and toxicity of DAG are dependent on maintenance of the plasma concentration of DAG above a threshold for given periods of time rather than on the total dose administered or on the total area under the plasma concentration × time curve. From the pharmacokinetic parameters determined in this study, one may calculate the amounts of DAG and rates of administration that would be necessary to achieve rapidly and thus maintain constant plasma concentrations for varying periods of time. Preclinical studies designed to determine the relationship of DAG concentration and duration of exposure on the colony-forming cells of human tumor cells are in progress as a prelude to additional clinical pharmacologic studies.

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# Phase I–II Study of Isotopic Immunoglobulin Therapy for Primary Liver Cancer[1,2]

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## SUMMARY

**A phase I–II study of isotopic immunoglobulin therapy was performed in 18 patients with primary liver cancer; 14 were evaluable for toxicity. The patients received a dose of 37–157 millicuries of $^{131}$I-labeled antibody. The dose-limiting factor appears to be hematologic toxicity, especially thrombocytopenia. An objective antitumor effect was seen in six of nine patients who were evaluable for response. Present results suggest that further clinical studies with isotopic immunoglobulin are indicated.**

[Cancer Treat Rep 66:289–297, 1982]

The demonstration that radiolabeled antibodies with specificity against tumor-associated antigens have successfully localized in primary tumors led to a phase I–II study using $^{131}$I-radiolabeled IgG directed against ferritin and CEA in an integrated treatment program for primary hepatic malignancies (cholangiocarcinoma and hepatoma) (1–4).

The present report reviews the physiologic effects and toxicity associated with the administration of the isotopic IgG.

## MATERIALS AND METHODS

### Antigens and Antisera

CEA and ferritin were isolated and purified, and antisera were produced by immunization of New Zealand White rabbits, as previously reported (5,6). Radiolabeled antiferritin was used in treating hepatoma, and anti-CEA was used in treating intrahepatic biliary carcinoma.

### Preparation of IgG

The IgG for patients 2, 5–8, 10, 12, and 14 (table 1) was prepared by two precipitations with ammonium sulfate at 33% saturation. The final IgG preparation was resuspended in 0.1 M sodium phosphate buffer (pH 7.6) plus 0.15 M NaCl (PBS) and was dialyzed against the same buffer overnight. This preparation showed a slight contamination with albumin (5%–10%) and β-globulins (5%), as determined by electrophoresis.

For the other patients who were given whole-immunoglobulin preparation (patients 1, 3, and 9), the IgG fraction of the antiserum was isolated by ion-exchange chromatography with DEAE cellulose (DE-52, Whatman, Ltd, England). The antiserum was dialyzed against 0.01 M phosphate buffer (pH 8.0) and was incubated with DE-52 equilibrated with the same buffer at a ratio of 1 ml of serum/5 g of cellulose for 1 hour at 4°C. The IgG was the only serum protein that did not bind to the cellulose at this ionic strength, and it was removed by suctioning through a Buchner funnel or by centrifugation. The IgG solution was dialyzed against PBS and concentrated by ammonium sulfate precipitation. All

[1]Received Mar 23, 1981; revised Aug 3, 1981; accepted Aug 31, 1981.

[2]This study was conducted in part by the Radiation Therapy Oncology Group (*Luther Brady, chairman*; CA-21661) and supported in part by Public Health Service grants CA-06973 and CA-29536 (The Johns Hopkins Oncology Center, Baltimore, MD) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and the Stuart and Barbara Janney Fund. Fairfax Hospital, Fairfax, VA, also participated in this study.

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TABLE 1.—Results of administering $^{131}$I-labeled antibody to patients with primary liver cancer*

| Patient No. | Age (yrs)/ sex | Dose of $^{131}$I (millicuries) | Wbc count nadir (cells × $10^3$/mm$^3$) | Platelet count nadir (cells × $10^3$/mm$^3$) | Other toxic effects | Objective response | Survival duration (mos) | Comments |
|---|---|---|---|---|---|---|---|---|
| 1 | 73/M | 37 | 4.4 | 51 | Low-grade fever | TETE | 5+ | Received high-specific-activity antibody |
| 2 | 40/F | 47 | 2.8 | 206 | | PR | 27 | |
| 3 | 70/M | 49 | 2.4 | 210 | Bacteremia (*Escherichia coli*), transient elevated LFTs | < 50% PR | 4.5+ | Prior partial hepatectomy and 5-FU and doxorubicin; no chemotherapy after RT |
| 4 | 45/M | 84 | 3.5 | 89 | Transient elevated LFTs | NE | 6 | Received antibody prepared by affinity chromatography |
| 5 | 74/M | 93 | 2.2 | 26 | | PR | 5 | |
| 6 | 35/M | 100 | 3.5 | 103 | Transient elevated LFTs | PR | 14 | |
| 7 | 49/F | 111 | 3.2 | 80 | Fever without source, transient elevated LFTs | NE | 6.5 | Prior partial hepatectomy |
| 8 | 17/M | 113 | 2.5 | 49 | Fever without source, transient elevated LFTs | PR | 6.5 | |
| 9 | 41/M | 117 | 0.84 | 12 | Hemorrhage (abdomen), transient elevated LFTs | NC | 5+ | Received high-specific-activity antibody |
| 10 | 70/F | 119 | 0.44 | 9 | Hemorrhage (GI tract), transient elevated LFTs | NC | 9 | No chemotherapy after RT |
| 11 | 69/M | 130 | 1.4 | 37 | Bronchospasm, transient elevated LFTs | NE | 9+ | Prior FAM. Received antibody prepared by affinity chromatography |
| 12 | 49/F | 150 | 0.4 | 11 | Hemorrhage (CNS), transient elevated LFTs | PR | 21+ | Prior FAM; no chemotherapy after RT |
| 13 | 59/F | 153 | 3.8 | 65 | Nausea and vomiting, hemorrhage (varices) | NE | 10+ | Received antibody prepared by affinity chromatography |
| 14 | 24/M | 157 | 2.1 | 33 | Transient elevated LFTs | PR | 10 | Prior hepatic artery ligation and 5-FU, doxorubicin, and bleomycin; no chemotherapy after RT |

*Patients 2, 5, and 10 had cholangiocarcinoma of the liver and received $^{131}$I-anti-CEA. All others had hepatoma and received $^{131}$I-antiferritin. TETE = too early to evaluate, + = alive, PR = partial response, LFTs = liver function studies, RT = radiation therapy, NE = not evaluable, NC = no change, and FAM = 5-FU, doxorubicin, and mitomycin.

samples were sterile-filtered with a 0.22-μ filter (Millipore Corp, Bedford, MA) before freezing.

Affinity column-purified antiferritin IgG, which was given to patients 4, 11, and 13, was prepared as described by Eveleigh (7). Purified ferritin was bound to CnBr-activated Sepharose 4B as described by the manufacturer (Pharmacia Fine Chemicals, Piscataway, NJ). Absorption of antibody and elution of specific antibody by 2 M ammonium thiocyanate were performed using an automated system (7). The eluted antibody was immediately dialyzed against PBS and concentrated by Amicon filtration followed by ammonium sulfate precipitation. Both DEAE-purified and affinity column-purified antibodies were free of contaminants as determined by electrophoresis, column chromatography, and polyacrylamide gel electrophoresis.

## Radioiodination of Antibody Preparation

The IgG fractions were labeled with $^{131}I$ by the lactoperoxidase method (8). In a sterile serum bottle, for every 2 mg of IgG, 10 μg of lactoperoxidase, 1 millicurie of $Na^{131}I$, and 10 μl of 8 mM hydrogen peroxide were added in that order. The mixture was stirred vigorously for 30 minutes at room temperature, and the labeled protein was separated from unbound label, using a Pharmacia PD-10, G-25 column (Pharmacia Fine Chemicals). Sterility and pyrogen testing of all samples was performed prior to administration.

Specific activities have recently been increased from 0.5 millicuries/mg of protein to approximately 10 millicuries/mg of protein. Affinity column-purified antibody was also labeled at the higher specific activity.

## Activity Testing of Antibody Preparations

Prior to labeling, the different antibody preparations were tested for activity by radioimmunoassay. The titer was defined as the amount of antibody necessary to precipitate 50% of a known quantity of $^{125}I$-labeled antigen (100 ng) in a double-antibody radioimmunoassay. For the ammonium sulfate-purified antibodies, approximately 90–100 ng of antibody was needed; for DEAE-purified antibody, 70–80 ng was needed; and for affinity column-purified antibody, 18–25 ng was needed.

Following radiolabeling, activities were measured by affinity chromatography. Approximately $1 \times 10^6$ counts/minute of labeled antibody was applied to a column containing 1.5 ml of affinity beads. Following washing with PBS, the specific antibody was eluted with 3 M ammonium thiocyanate. The activity was defined as the percent of activity specifically bound and eluted from the column. Normal IgG routinely bound $< 5\%$, ammonium sulfate- or ion-exchange-purified antibody bound 18%–22%, and affinity column-purified antibody bound 68%–74%.

All antibody preparations were negative by bacteriology and pyrogenicity testing.

## Patient Selection and Therapy

All patients selected for the isotopic IgG therapy had histologically confirmed, nonresectable primary liver cancer (hepatoma or cholangiocarcinoma). Patients were required to have measurable or evaluable disease. All patients who were given the radiolabeled IgG were required to be capable of self-care and to have a Karnofsky performance status $\geq 60\%$. Adequate pretreatment renal (BUN $\leq 25$ mg/100 ml and creatinine $\leq 1.5$ mg/100 ml), bone marrow (wbc count $\geq 4000/mm^3$ and platelet count $\geq 100{,}000/mm^3$), cardiac (ECG without arrhythmia or major ischemic changes), and pulmonary (forced expiratory volume, total lung capacity, or diffusing capacity of carbon monoxide $> 50\%$ of predicted value) functions were required. Patients had to be $< 75$ years of age and clear of active infection, with no history of allergy (asthma or eczema). All patients entered in the study gave informed written consent.

Although this report will evaluate the effects of administering isotopic IgG, its administration was part of a therapeutic program for primary liver cancer. The multimodality treatment program consisted of 2100 rad of external-beam irradiation integrated with alternating doxorubicin (15 mg) and 5-FU (500 mg), both given iv. In some of the first patients studied, this was followed by 6 $g/m^2$ of metronidazole, a known radiosensitizer, but metronidazole was discontinued to reduce the complexity of the protocol when the program became a Radiation Therapy Oncology Cooperative Group study. One month after completion of this part of the therapy, patients received two monthly cycles of doxorubicin (50 $mg/m^2$) iv plus 5-FU (600 $mg/m^2$) iv. One month following these two cycles, the patients received the appropriate isotopic IgG in escalating doses (table 2).

IgG was administered at least 4 weeks after the last therapy since, in most instances, any toxicity or antitumor response due to the prior treatment would have been noted by that time. The initial dose of $^{131}I$-labeled IgG was approximately one third to one fourth the dose used in the treatment of thyroid cancer (9).

TABLE 2.—Phase I–II study of isotopic IgG in an integrated multimodal treatment program for primary liver cancer*

| Day of therapy | RT† | CT† |
|---|---|---|
| 1 | x | x |
| 2 | x | |
| 3 | x | x |
| 4 | x | |
| 5 | x | x |
| 6 | x | |
| 7 | x | x |

*Effects of this therapy were evaluated after 1 mo; patients were then given doxorubicin (50 mg/m² iv) + 5-FU (600 mg/m² iv) q 28 days × 2. After tumor evaluation, 1 mo later, patients were given isotopic IgG. Toxicity and tumor evaluations were performed weekly for 1 mo, then monthly or as clinically indicated.

†RT = radiation therapy (300 rad); CT = chemotherapy (15 mg of doxorubicin iv + 500 mg of 5-FU iv).

## Tumor and Toxicity Evaluation

Prior to the initiation of therapy, a complete evaluation was performed, which consisted of a history and physical examination, cbc, urinalysis, liver function studies, chest roentgenograms, pulmonary function studies, ECG, and measurement of electrolytes, BUN, creatinine, calcium and phosphorus, creatine phosphokinase, $T_3$, $T_4$, CEA, ferritin, and alpha-fetoprotein.

The radiolabeled IgG was administered under very close medical observation for 2 hours, and then the patient was observed more routinely. While in the hospital, the patient took his own temperature and pulse and recorded his intake and output. A weekly evaluation included a history and physical examination, performance status, cbc, renal and liver function studies, urinalysis, and measurement of electrolytes and creatine phosphokinase.

Liver scans and computerized axial tomographic (CAT) scans of the liver were used in evaluating normal parenchyma and tumor response. Tumor volume calculations were measured from sequences of CAT scan slices of the liver. The volume determinations made by this method appear to be accurate and reproducible (10). A complete response was defined as complete disappearance of all clinically detectable tumor. A response was defined as partial if the tumor volume in the liver was reduced by at least 50% or if the liver was felt ≥ 5 cm below the right costal margin and subsequently decreased in size by ≥ 30%. A < 50% decrease or a < 25% increase in the tumor volume was considered to be no response. Progression was defined as an increase in the tumor volume by > 25% or the appearance of new areas of malignant disease.

## Preparative Regimens and Specialized Room Requirements

All patients who received the isotopic IgG received two drops of saturated solution of potassium iodide or ten drops of Lugol's solution twice a day orally, beginning 7 days prior to the radiolabeled antibody administration and for at least 2 weeks following the treatment. The patients were hospitalized in a room protected by portable shields (designed by Radium Chemical Company and The Johns Hopkins Radiation Oncology Physics Section) to minimize the exposure rate to < 2 milliroentgens/hour on the outside of the shielding. The patients could be viewed from a lead window in the shielding or by a larger mirror behind it. Skin testing with radiolabeled antibody and eye testing with unlabeled antibody were performed 1 day prior to the radiolabeled antibody administration. Independent laboratory verification of sterility and lack of pyrogenicity of the radiolabeled antibody was confirmed before administration. The patients had an iv catheter line with a three-way stopcock for administration of radiolabeled antibody. All patients were taught temperature recording as well as recording of intake and output.

## Radioactivity Monitoring

The effective half-life of $^{131}I$-labeled antibody for total-body activity was determined from serial measurements of radioactivity in blood samples by scintillation counting (counts/minute/ml of blood) and by monitoring the $^{131}I$ exposure rate at the bedside with an unshielded Geiger counter at a distance of 1 m from the patient. Measurements were started 1 day following the radioimmunoglobulin infusion; they were recorded daily for the duration of the patient's hospitalization.

Measurements of the uptake, decay, and activity of $^{131}I$ circulating in the whole body and deposited in the liver and tumor tissue were recorded as previously reported (11).

# RESULTS

## Analysis of Toxicity

Of 37 patients entered in the study, 18 have received the isotopic IgG. Fourteen were evaluable for toxicity, four were too recently treated for the present evaluation (table 1), nine are in the early part of the multimodality treatment program and have not yet received the isotopic IgG, and ten did not receive the isotopic IgG because of rapid disease progression leading to a reduced performance sta-

tus or death. Five of the fourteen evaluable patients who received isotopic IgG had failed prior therapy before being entered in the study. Patients 11 and 12 had received 5-FU, doxorubicin, and mitomycin; patient 3 initially had had a partial hepatectomy and had received 5-FU and doxorubicin; patient 14 had had a hepatic artery ligation and had received 5-FU, doxorubicin, and bleomycin; and patient 7 had had a partial hepatectomy. Patients 3, 12, and 14 did not receive chemotherapy following the liver radiation, prior to receiving the isotopic IgG. Patient 10 did not receive the chemotherapy because she had had a prolonged aplasia following the liver irradiation. Of the evaluable patients, nine were men and five were women. The average age was 49 years (range, 17–74).

Of the fourteen evaluable patients, three received 37, 47, and 49 millicuries of $^{131}$I-labeled antibody, seven received 100 ± 19 millicuries, and four received 150 ± 20 millicuries.

Following the administration of isotopic IgG, patients were evaluated for both early and late toxic effects, based on Eastern Cooperative Oncology Group criteria. The toxicity was assessed on a six-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening, and 5 = drug-related death). Table 3 summarizes the toxicity data by type and degree.

It was clear that hematologic toxic effects (usually leukopenia and thrombocytopenia) were the most prevalent, occurring to a severe or worse degree in 50% (seven of 14) of the patients (figs 1 and 2). The median wbc count was 840/mm$^3$, occurring during Week 6 after therapy, while the median platelet count was 25,000/mm$^3$, occurring during Week 4.

Table 4 gives the distribution of patients by their worst toxic effects.

Four patients (Nos. 9, 10, 12, and 13) (29%) experienced life-threatening complications following the administration of the radiolabeled isotopic IgG. Patients 9, 10, and 12 had complications that were due to both thrombocytopenia and leukopenia, with platelet count nadirs (< 25,000/mm$^3$) occurring in Weeks 4, 4, and 6 and wbc count nadirs (< 1000/mm$^3$) occurring in Weeks 5, 6, and 6, respectively. All three patients received platelet as well as rbc transfusions, and all required antibiotic therapy even though no localized source of infection was found. These patients developed a hemorrhagic diathesis associated with marked thrombocytopenia; one had a subdural hematoma, another had upper gastrointestinal bleeding, and the third had bleeding into

TABLE 3.—Isotopic IgG-related toxic effects

| | Degree* | | | | | |
|---|---|---|---|---|---|---|
| Toxic effect | None | Mild | Moderate | Severe | Life-threatening | Lethal |
| Anemia | 6 | 3 | 1 | 4 | 0 | 0 |
| Hemorrhage | 11 | 0 | 0 | 0 | 3 | 0 |
| Thrombocytopenia | 2 | 1 | 4 | 4 | 3 | 0 |
| Leukopenia | 0 | 6 | 4 | 1 | 3 | 0 |
| Infection | 9 | 0 | 1 | 1 | 3 | 0 |
| Gastrointestinal | | | | | | |
| Hepatic | 4 | 7 | 3 | 0 | 0 | 0 |
| Nausea and vomiting | 13 | 0 | 1 | 0 | 0 | 0 |
| Other | 14 | 0 | 0 | 0 | 0 | 0 |
| Pulmonary | 14 | 0 | 0 | 0 | 0 | 0 |
| Cardiac | 14 | 0 | 0 | 0 | 0 | 0 |
| Neurologic | | | | | | |
| Peripheral | 14 | 0 | 0 | 0 | 0 | 0 |
| CNS | 14 | 0 | 0 | 0 | 0 | 0 |
| Genitourinary | 14 | 0 | 0 | 0 | 0 | 0 |
| Allergy | 13 | 0 | 0 | 1 | 0 | 0 |
| Fever | 6 | 0 | 8 | 0 | 0 | 0 |
| Skin and mucosa | 14 | 0 | 0 | 0 | 0 | 0 |

*Values = No. of patients.

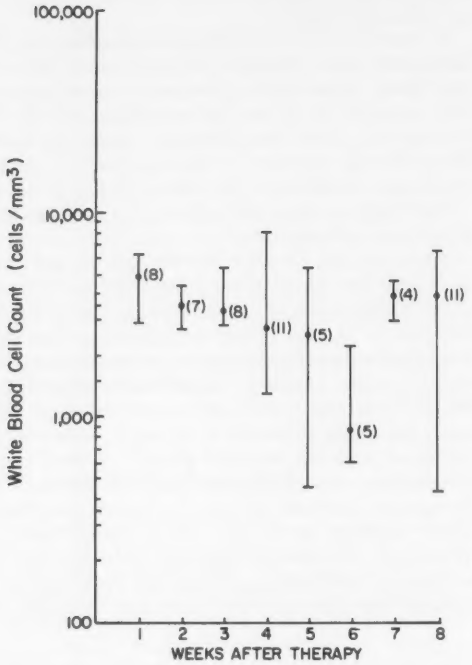


FIGURE 1.—Effect of isotopic IgG therapy on wbc count; • = median, I = range, values in parentheses = No. of patients evaluated.

the abdomen. The fourth patient with a life-threatening toxic effect (No. 13) hemorrhaged from esophageal varices and required rbc transfusions during Week 2 following the administration of the isotopic IgG.

Patients 5, 11, and 14 experienced severe toxic effects. Patients 5 and 14 had severe thrombocytopenia, with platelet count nadirs of 26,000 and 33,000/mm³, respectively, occurring 4 weeks after therapy. These patients did not require platelet transfusions. Patient 11 had bronchospasm approximately 1 hour following the administration of the radiolabeled antibody, requiring parenteral medication for relief.

Moderate nausea and vomiting occurred in one of 14 patients (No. 13) approximately 1–2 hours after the isotopic IgG therapy. Eight of 14 patients (57%) had fever (> 38°C to < 40°C). In patients 9–12, the fever was attributed to the severe or life-threatening leukopenia. In patient 3, who had a wbc count of 2400/mm³ 1 week after therapy, a fever was associated with a positive blood culture (*Escherichia coli*), which cleared with the administration of appropriate antibiotics. Patients 7 and 8 had fever without an apparent etiology several weeks after therapy. Two days after the isotopic IgG administration, patient 1 developed a low-grade fever, which persisted for 1 week; a complete fever workup was negative for infection, although the patient did receive tetracycline, with resolution of the fever within 1 week.

Transient hepatic enzyme elevation occurred in ten of the 14 patients; this usually occurred several weeks following the therapy.

No cardiac, pulmonary, or renal toxicity could be identified.

TABLE 4.—Distribution of patients by worst toxic effect

| Degree of toxic effect | No. of patients (%) |
|---|---|
| None | 0 (0) |
| Mild | 4 (29) |
| Moderate | 3 (21) |
| Severe | 3 (21) |
| Life-threatening | 4 (29) |
| Lethal | 0 (0) |
| Total | 14 (100) |

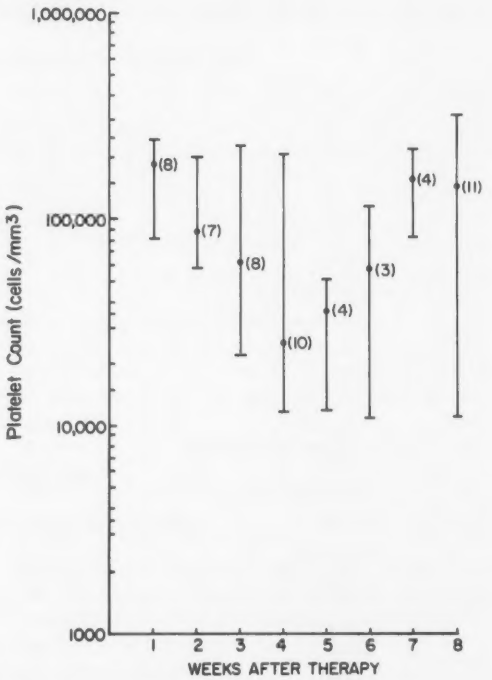


FIGURE 2.—Effect of isotopic IgG therapy on platelet count; • = median, I = range, values in parentheses = No. of patients evaluated.

## Dosimetry

Dosimetric studies have been previously reported for four hepatoma patients treated with $^{131}$I-labeled antiferritin (93–157 millicuries) (11). Studies included liver and tumor volume calculations from sequences of CAT scan slices, in vivo quantitation of radiolabeled antibody in the liver and tumor, determination of kinetic parameters of radioimmunoglobulin for liver, tumor, and the total body, and computation of dose rates (rad/hour) and total dose (rad) for these tissues. Tumor volumes for these patients prior to the administration of radiolabeled antibody ranged from 370 to 920 cm$^3$ and liver volumes ranged from 900 to 1980 cm$^3$. In vivo quantitation, performed 4–8 days following infusion of $^{131}$I-labeled antiferritin, demonstrated tumor:liver ratios of about 2:1 for the deposition of radioimmunoglobulin in these tissues. Mean values for the effective half-life for radiolabeled antibody in the liver and tumor and for total-body activity were 7.4 and 3.6 days, respectively. For patients 6, 14, 5, and 8, the $^{131}$I radiation doses for tumor tissue were 1500, 1800, 1900, and 2200 rad, respectively; for liver tissue, they were 400, 1000, 600, and 900 rad, respectively; and for total-body irradiation (TBI), they were 110, 220, 140, and 200 rad, respectively (11).

## Therapeutic Response

Of the fourteen patients who received the radiolabeled isotopic IgG, nine were evaluable for response; there were six partial responses. In patient 5, the liver was felt ≥ 5 cm below the costal margin prior to therapy and subsequently decreased in size by ≥ 30%; CAT scans were not available. In patient 3, who had an objective response, CAT scan showed a 40% decrease in tumor volume, which was associated with a clinical response. Figures 3 and 4 show CAT scans of the liver in patient 12 before and 1½ years after treatment.

FIGURE 3.—CAT scan of the liver of patient 12 prior to treatment with 150 millicuries of $^{131}$I isotopic IgG.

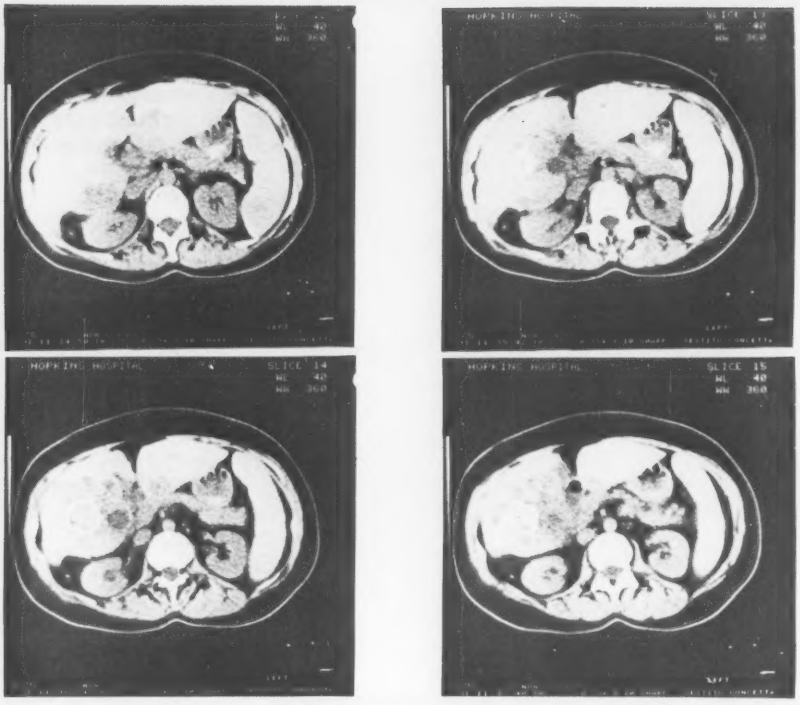

FIGURE 4.—CAT scan of the liver of patient 12 performed 1½ years after treatment with isotopic IgG. Note ring of calcification in the liver where tumor had been present.

## DISCUSSION

When the clinical use of the radiolabeled isotopic immunoglobulin began, there were two concerns: (*a*) that immune complexes (antigen and antibody) would be formed in tissues, causing extensive injury, in particular, renal damage, and (*b*) that isotopic immunoglobulin would deposit in the bone marrow due to the presence of normal ferritin, causing irreversible aplasia.

There has been no evidence of the formation of detrimental immune complexes in any of the 14 patients who received the radiolabeled isotopic IgG, although one patient, a 69-year-old white male with a hepatoma, who received 130 millicuries of $^{131}$I-labeled antiferritin, developed transient bronchospasm 1 hour after its administration, which rapidly responded to appropriate therapy. This patient had received a tracer dose of IgM monoclonal antiferritin for scanning 48 hours prior to the therapeutic administration of the polyclonal antibody.

Fever as a manifestation of an allergic reaction to the radiolabeled isotopic IgG was not evident, although patient 1 did develop a low-grade fever 48 hours after therapy. The exact cause of the temperature elevation could not be determined, although the fever disappeared after the patient was empirically given antibiotic therapy. Five of the other febrile episodes were attributable to presumed or proven infection associated with leukopenia, while two others occurred several weeks after isotopic IgG therapy, without a readily apparent etiology.

The dose-limiting factor of the radiolabeled IgG appeared to be hematologic toxicity, particularly thrombocytopenia due to TBI of free-circulating radiolabeled antibody. There was no binding of radiolabeled antibody by the bone marrow ferritin, as that would have been demonstrable by nuclear scanning and was not. In fact, in a patient with multiple myeloma, a ferritin-containing tumor, only the involved bones scanned as positive (2).

The hematologic toxicity appeared to be dose-related to the amount of radiolabeled IgG administered. An estimated dose of TBI was approximately 1 rad/millicurie of radioimmunoglobulin. If the amount of radiolabeled isotopic IgG given is

divided into 50, 100, and 150 millicuries for comparison purposes, the median wbc count nadirs are 2.8, 2.5, and 1.8 × $10^3$ cells/$mm^3$, respectively, and the median platelet count nadirs are 178, 49, and 35 × $10^3$ cells/$mm^3$. There were probably other factors contributing to this hematologic toxicity. Some patients had failed previous treatment protocols with chemotherapy, and bone marrow reserve was affected in other patients who received the chemotherapy and the radiation therapy to the liver. In addition, the effective half-life of the $^{131}$I-labeled antibody could be prolonged if a patient had a "third space" loss, in particular, ascites. In four patients with hepatoma, the average effective half-life of I-labeled antibody was 3.6 days. Patient 12 had documented ascites, and the prolonged effective half-life of $^{131}$I-labeled antibody was 5.6 days, thereby contributing more TBI. Her wbc count nadir was 400/$mm^3$ and her platelet count nadir was 11,000/$mm^3$.

No other organs seemed to be affected adversely by the administration of the isotopic IgG, although liver function studies showed transient elevations in 72% of the patients, probably due to acute radiation effects.

The present study has demonstrated that the major toxicity of the radiolabeled antibody is hematologic. This toxicity may be reduced by decreasing the administered dose, increasing the specific activity (isotope-antibody ratios), fractionation of isotopically labeled antibody, and possibly by increasing specificity by using monoclonal antibody while reducing the total dose administered.

Our group of investigators hopes to establish a dose-response curve for radioimmunoglobulin administration in hepatoma. Initially, sequential, increasing doses of radioimmunoglobulin were administered. It was determined that such administration did not increase the dose achieved at the tumor site and that, therefore, the dose administered would not establish a dose-response curve (11). Development of sophisticated techniques to evaluate tumor volume and correlate dose deposition with volumetric configuration has allowed for the more precise determination of the dose achieved at the tumor site. Studies of the dose-response will be dependent on these calculations in future patients.

Further studies have demonstrated that a smaller fractional dose of radioimmunoglobulin could be given in sequence and achieve potentially more radiation at the target volume (unpublished data); for example, it has been determined that 30 millicuries of radiolabeled antiferritin immunoglobulin saturated the hepatomas and that a dose > 30 millicuries in a single administration only contributed further to TBI. Depending on the effective half-life of the radiolabeled immunoglobulin, a second or third administration would amplify the total dose achieved at the tumor site. This work is presently under way.

For more conclusive results on the therapeutic activity of isotopic immunoglobulin, study of additional patients and more prolonged observation are needed; however, there is a suggestion that therapy using isotopic immunoglobulin as a potential antineoplastic agent is reasonable.

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# Phase II Trial of Combination Therapy With Continuous-Infusion PALA and Bolus-Injection 5-FU[1]

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## SUMMARY

**Fifty-seven patients were treated with continuous infusion of PALA at a dose of 850 mg/m²/day × 5 and iv bolus injection of 5-FU at a dose of 300 mg/m²/day × 5, which was given at the end of each 24-hour PALA infusion; the treatment interval was 28 days. The overall response rate among 43 evaluable patients was 14%. Two of 21 patients (10%) with colorectal carcinoma, two of eight patients with pancreatic carcinoma, and two of four patients with breast carcinoma achieved partial responses lasting 2–12 months. One of the responding patients with colon cancer and two with breast cancer had failed to respond to prior therapy with 5-FU; one of the responding patients with breast cancer had previously received an inadequate trial of a similar regimen. Toxic effects were mild to moderate and were confined to oral mucositis in most patients. It is concluded that this regimen offers little enhancement of the antitumor activity of 5-FU in patients with colorectal cancer previously treated with 5-FU. Investigation of other infusion techniques for the PALA and 5-FU combination is recommended, with particular attention to the treatment of colorectal, pancreatic, and breast carcinomas.**

[Cancer Treat Rep 66:299–303, 1982]

Experimental evidence indicates that two biochemical mechanisms may be responsible for the antitumor activity of 5-FU: (*a*) 5-FU is converted to FUTP, which is incorporated into RNA, and (*b*) 5-FU is converted to FdUMP, which binds irreversibly to thymidylate synthetase, thereby inhibiting synthesis of dTMP and DNA (1,2). Several studies suggest, however, that antitumor activity correlates with the incorporation of FUTP into RNA and not with the inhibition of DNA synthesis (3–5).

PALA is a transition-state analog inhibitor of aspartate transcarbamylase, the second enzyme involved in de novo pyrimidine biosynthesis (6). This agent is active against animal tumor models when used alone and in combination with 5-FU (R. Johnson, personal communication) (7). PALA reduces uracil nucleotide pools (8), increases the inhibition of thymidylate synthetase, and enhances the incorporation of 5-FU into RNA (5,9–11). This increase in (5-FU)RNA formation is associated with increased in vitro antitumor activity.

We have recently completed a phase I evaluation of PALA administered as a 5-day continuous infusion (12). Dose-limiting toxicity was observed at a dose ≥ 1200 mg/m²/day × 5. Diarrhea, skin rash, and oral mucositis were the major toxic effects at these doses. No significant antitumor responses were noted among 15 evaluable patients.

In a phase I evaluation of 5-FU in combination with PALA, dose-limiting toxicity was observed with continuous infusion of PALA at a dose of 940 mg/m²/day × 5 and with iv bolus injection of 5-FU at a dose of 345 mg/m²/day × 5 every 4 weeks (13). Each daily bolus of 5-FU was given at the end of each 24-hour PALA infusion. Toxic effects included moderately severe oral mucositis, diarrhea, and

[1]Received Apr 1, 1981; revised July 6, 1981; accepted July 24, 1981.

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TABLE 1.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Total | 57 |
| Evaluable for response | 43 |
| Age (yrs) | |
| Mean: 53 | |
| Range: 19–76 | |
| Prior treatment | |
| Chemotherapy | 21 |
| Radiotherapy | 4 |
| Chemotherapy and radiotherapy | 8 |
| Fluorinated pyrimidine | 22 |
| None | 10 |
| No. of courses of therapy | |
| Total: 167 | |
| Evaluable for toxicity: 161 | |

myelosuppression. Objective responses were observed in large cell carcinoma of the lung, fibrous histiocytoma, and adenocarcinoma of the colon. The objective responses to PALA and 5-FU occurred at doses resulting in mild to moderate toxicity. These findings suggested that this combination should undergo phase II evaluation.

## MATERIALS AND METHODS

Patients entered in this study had histologically confirmed malignancy. All patients had measurable disease refractory to standard therapy, had a performance status of $\leq 2$ (Eastern Cooperative Oncology Group scale), and had fully recovered from the toxic effects associated with prior treatment. Informed consent was obtained prior to therapy.

PALA was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The drug was prepared and stored as previously described (13). 5-FU was obtained from The Upjohn Co, Kalamazoo, MI.

PALA was given at a dose of 850 mg/m²/day × 5 by continuous infusion, and 5-FU was given at a dose of 300 mg/m²/day × 5 by iv bolus injection after each 24-hour PALA infusion. The treatment interval was 28 days. Based on our phase I toxicity data for the combination of PALA and 5-FU, the selected dose levels would be expected to induce tolerable toxic effects. In subsequent courses, the daily dose of each agent was reduced by 10%, or the duration of a subsequent course was shortened by 1 day (20% dose reduction) if patients experienced moderately severe oral mucositis (unable to tolerate semisolid food) for $\geq 7$ days or developed unacceptable myelosuppression (wbc count $< 1000/mm^3$ or platelet count $< 50,000/mm^3$).

Prior to the initiation of chemotherapy, all patients had a complete history and physical examination. Laboratory evaluation included cbc with differential, platelet count, liver function tests, and measurement of BUN, creatinine, serum electrolytes, glucose, and 24-hour creatinine clearance. All measurable disease was documented prior to the first course of therapy. Cbc, differential, and platelet count were repeated weekly. Liver function tests, cbc, differential, platelet count, and measurement of BUN and creatinine were repeated prior to each course of therapy.

## RESULTS

Fifty-seven patients eligible for the study received 167 courses of therapy (table 1); 43 patients who received two or more courses of therapy were considered evaluable for antitumor response. The remaining 14 patients died of malignant disease after one course of therapy or before sufficient time had elapsed to permit evaluation for response. One patient died of causes related to toxic effects of ther-

TABLE 2.—Therapeutic response*

| Diagnosis | No. of patients | Partial response | No response | Prior 5-FU/ FUDR therapy |
|---|---|---|---|---|
| Carcinoma | | | | |
| Colorectal | 21 | 2 | 19 | 16 |
| Pancreatic | 8 | 2 | 6 | 0 |
| Breast | 4 | 2 | 2 | 4 |
| Sarcoma | 4 | 0 | 4 | 0 |
| Other | 6 | 0 | 6 | 2 |

*Partial response = > 50% decrease in the sum of the products of perpendicular diameters of measurable lesions that persists for $\geq 1$ mo; complete response = disappearance of all detectable evidence of disease for $\geq 1$ mo; and no response = < 50% decrease in the sum of the products of perpendicular diameters of measurable lesions, stable disease, disease progression, or response lasting < 1 mo.

apy (*see* below). The characteristics of the evaluable patients are listed in table 1.

The overall objective response rate was 14% (table 2). Two of 21 patients (10%) with colorectal carcinoma experienced partial responses of 4 and 6 months. One patient responded with regression of pulmonary nodules; the other patient underwent exploratory laparotomy, which showed a > 50% reduction of a local recurrence of colorectal carcinoma and the resolution of hydronephrosis. Both responding patients had been previously treated with 5-FU; one failed to respond to the drug and was considered resistant to it, and 5-FU therapy was discontinued in the other because of intolerable toxicity. Two of eight patients with previously untreated pancreatic carcinoma also responded for 6 and 12 months; a partial response in one patient was confirmed at laparotomy, and abdominal skin nodules decreased in the other. Two of four patients with breast carcinoma achieved a partial response; a chest wall mass resolved in one, and an axillary node and chest wall nodule regressed in the other. The durations of these responses were 2 and 3 months, respectively. Both patients had previously received 5-FU in combination chemotherapy programs without response; in one of these patients, the trial of a regimen similar to ours was inadequate. No response was noted in four evaluable patients with soft tissue sarcomas. No additional responses were observed in six patients with ovarian papillary cystadenocarcinoma, cholangiocarcinoma, large cell carcinoma of the lung, adenocarcinoma of the duodenum, squamous carcinoma of the lung, and gastric carcinoma.

Fifty-four patients received 161 courses of therapy that were evaluable for toxic effects. In most patients, toxic effects were mild to moderate and were confined to oral mucositis (table 3). Hematologic toxicity was observed infrequently. Diarrhea, an erythematous plaquelike skin rash, and myelosuppression were observed in order of decreasing frequency.

Oral mucositis occurred in 90 courses of therapy (56%) and hospitalization was required for iv hydration in three patients. Mucositis usually began on Days 5–8 of treatment and persisted for 7–14 days. Diarrhea occurred in 66 courses of therapy (41%). In two courses, the diarrhea was bloody but self-limited. Diarrhea generally coincided with more severe oral mucositis but occasionally developed independently. The erythematous plaquelike skin rash was noted in 11 courses of treatment (7%) and was mild in all but two cases. The rash was associated with minimal symptomatology, and except for one case, healed prior to the initiation of the next cycle of treatment.

Myelosuppression was unusual. Of 161 courses, only 14 (9%) were complicated by leukopenia (wbc count < 3000/mm$^3$) and six (4%) were complicated by thrombocytopenia (platelet count < 100,000/mm$^3$). The platelet count nadir occurred between Days 9 and 28 (median day, Day 18). Thrombocytopenia was sufficiently severe to require platelet transfusion in one patient. Three instances of leukopenia were accompanied by febrile illnesses requiring hospitalization and antibiotics. One patient developed a wbc count of 400/mm$^3$ and died of infection secondary to aspiration pneumonia. This patient's death was considered to be related to toxicity of therapy. Myelosuppression coincided with the appearance of oral mucositis.

Eighteen patients required reduction of their daily treatment dose by 10% or shortening of their therapy by 1 day in 59 courses (36%). Dose reduction was judged necessary when oral mucositis was moderately severe or when myelosuppression was severe (wbc count < 1000/mm$^3$ or platelet count < 50,000/mm$^3$).

## DISCUSSION

An objective partial response was observed in six of 43 evaluable patients treated with this combination; the overall response rate was 14%. Patients with colorectal carcinoma were treated in sufficient numbers to evaluate antitumor activity; the response rate was 10%. Of 16 patients with colorectal

TABLE 3.—Toxicity

| | Degree* | | | | |
|---|---|---|---|---|---|
| Toxic effect | 0 | 1+ | 2+ | 3+ | 4+ |
| Mucositis† | 71‡ | 26 | 33 | 27 | 4 |
| Diarrhea | 95 | 26 | 31 | 9 | 0 |
| Rash | 150 | 9 | 0 | 1 | 1 |

*Based on Eastern Cooperative Oncology Group scale of graded toxicity.
†Oral mucositis was considered to be dose-limiting if the degree of toxicity was ≥ 3+ and persisted for ≥ 7 days. Neither diarrhea nor rash was dose-limiting.
‡Values = No. of courses.

carcinoma previously treated with 5-FU or 5-FUDR, two who were considered either resistant to or intolerant of the agents had objective responses to the PALA and 5-FU combination. Four other responses were observed, two each in patients with pancreatic and breast carcinomas. The responding patients with pancreatic carcinoma had not received prior 5-FU. One of the responding patients with breast carcinoma had failed to respond to prior 5-FU in combination chemotherapy, and the other patient had received an inadequate trial of a similar regimen.

The objective response data suggest that PALA can enhance the antitumor activity of 5-FU in certain patients. PALA re-established the activity of 5-FU against one colorectal tumor resistant to 5-FU alone. A similar pattern of response was documented in at least one and possibly two patients with breast carcinoma. PALA and 5-FU also induced tumor regression in two of eight previously untreated patients with pancreatic carcinoma. Treated malignancies of other histologic types were resistant to this combination. Thus, PALA may enhance the activity of 5-FU against tumors regarded as sensitive to this agent.

The PALA and 5-FU combination was effective in two previously untreated patients with pancreatic carcinoma. This combination may deserve further exploration as a phase II study in the treatment of pancreatic tumors. Although responses were also observed among patients with colorectal carcinomas who had received extensive prior exposure to 5-FU, the response rate is inferior to that (34%) observed in patients with colorectal carcinoma previously unexposed to fluorinated pyrimidines and treated with escalating doses of 5-FU (14). Thus, this regimen of PALA and 5-FU offers too little enhancement of antitumor activity to sanction its broad usefulness in patients who have received 5-FU. Possible reasons for tumor resistance to the drug combination include: (*a*) failure to achieve, in vivo, the drug levels necessary to produce biochemical effects; (*b*) elevated levels of aspartate transcarbamylase; and (*c*) use of pyrimidine salvage pathways (15). Subsequent studies should explore pharmacologic monitoring of the inhibition of aspartate transcarbamylase; the incorporation of 5-FU into tumor cells, bone marrow, and oral mucosa; and the blood levels of the infused agents during and after treatment.

The biochemical rationale for the combination of PALA and 5-FU is based on the enhancement of the incorporation of 5-FU into tumor cell RNA, thereby increasing the antitumor response. As RNA synthesis continues throughout the cell cycle, cellular exposure to 5-FU should be continuous to maximize this effect. In a randomized treatment of colorectal carcinoma, comparison of a continuous 120-hour infusion of systemic 5-FU versus bolus injection daily for 5 days demonstrates a higher response rate and less toxicity for the continuous-infusion regimen (16). Since administration by continuous infusion should be more effective in enhancing 5-FU incorporation into tumor cell RNA, it may be more relevant to consider the effects of PALA with 5-FU given by continuous infusion. In a recent study of continuous infusion of PALA and 5-FU, evidence was presented that suggested an enhancement of the biochemical and antitumor effects (10).

In summary, this dose schedule of PALA and 5-FU has acceptable gastrointestinal and hematologic toxicity while demonstrating effectiveness against certain breast and colorectal carcinomas previously resistant to 5-FU. The drug combination might be evaluated in the treatment of patients with advanced colorectal carcinoma not previously exposed to 5-FU and compared to the best available treatment with 5-FU alone. Further, this study suggests some activity of the drug combination against pancreatic carcinoma in previously untreated patients. Dose schedules of this combination are currently being explored using continuous infusion of both agents.

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# Vindesine in the Treatment of Squamous Cell Carcinoma, Adenocarcinoma, and Large Cell Carcinoma of the Lung[1,2,3,4]

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## SUMMARY

**The antineoplastic activity of vindesine was evaluated in a phase II trial in patients with squamous cell carcinoma, adenocarcinoma, and large cell carcinoma of the lung. Sixty-three patients participated in the trial: 22 with squamous cell carcinoma, 25 with adenocarcinoma, and 16 with large cell carcinoma. Most of the patients had not received prior treatment; six had received single-agent chemotherapy. The dose of vindesine was 4 mg/m² every 2 weeks, reduced or increased according to hematologic and neurologic side effects. Fifty-four patients were evaluable. Objective response was observed in two of 19 patients with squamous cell carcinoma (11%), in six of 22 patients with adenocarcinoma (27%), and in one of 13 patients with large cell carcinoma (8%). In patients with adenocarcinoma the median duration of response was 196 days (range, 71–450+). Twenty-five percent of the evaluable patients received ≥ 100% of the scheduled dose. In 39% of the patients, dose reductions were necessary because of leukopenia, and in 63%, dose modifications were necessary because of neurotoxic effects. It is concluded that vindesine as a single-agent chemotherapy is active against adenocarcinoma of the lung. Activity in squamous cell carcinoma and large cell carcinoma appears to be less, but not totally absent.**

[Cancer Treat Rep 66:305–309, 1982]

Although vindesine is the most recent vinca alkaloid to undergo clinical investigation, therapeutic activity has already been demonstrated in various hematologic malignancies and solid tumors in which vinblastine and/or vincristine are active, including cases with clinical resistance to these two compounds (1–4).

Vindesine has well-documented antitumor activity against small cell carcinoma of the lung (5,6), and recent studies (7–9) have also indicated clinical antineoplastic activity of vindesine in the treatment of other major histologic types. This report contributes additional data concerning the activity of vindesine in the treatment of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma of the lung.

## MATERIALS AND METHODS

Patients were eligible for the trial if they had an unresectable pulmonary tumor detected radiologically and proven to be squamous cell carcinoma, adenocarcinoma, or large cell carcinoma according to the World Health Organization (WHO) classification (10). A diagnostic biopsy was obtained from a bronchoscopy, mediastinoscopy, thoracotomy, percutaneous needle, or scalene node biopsy.

All patients were < 75 years of age, with an

[1]Received Apr 10, 1981; revised June 19, 1981; accepted June 30, 1981.

[2]Supported by Olga and Esper Boel's Foundation.

[3]Presented in part at Lilly's International Vinca Alkaloid Symposium, Frankfurt/Main, W Germany, Nov 1980.

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TABLE 1.—Criteria for dose modifications of vindesine

| Dose* | Toxic effects | |
|---|---|---|
| | Wbc count (cells/mm³) | Platelet count (cells/mm³) |
| 125† | ≥ 4000 | >100,000 |
| 100 | ≥ 3000 | ≥100,000 |
| 50 | > 2000–3000 | > 50,000–100,000 |
| 0 | ≤ 2000 | ≤ 50,000 |
| 50 | Constipation (3 days) | |
| 50 | Pain (abdominal/jaw) | |
| 50 | Paresthesias (disabling) | |
| 50 | Weakness (walking distance, 30 m) | |
| 0 | Continued neurotoxicity in spite of dose reductions | |

*Values = % of scheduled dose.

†This dose was given only if patients had little or no neurotoxic effects.

expected survival time of at least 4 weeks. Initiation of vindesine therapy was deferred until hematologic restitution following chemotherapy. The wbc and platelet counts were at least 3000 and 100,000/mm³, respectively, unless bone marrow metastases were present.

The neoplastic disease was evaluated in all patients. Clinical examinations were performed before therapy and every 2 weeks thereafter, while hemoglobin concentration and wbc and platelet counts were performed every week. Serum electrolytes, creatinine, alkaline phosphatase, LDH, and bilirubin were measured after 4 weeks and then every 6–8 weeks. Chest radiography was performed every 4 weeks.

Vindesine was administered iv by bolus injection at a dose of 4 mg/m²/week × 8, followed by 4 mg/m² every 2 weeks. The criteria for dose reduction and increase are summarized in table 1. Vindesine treatment was continued until disease progression was detected.

Response in patients who died within the first 2 weeks was regarded as unevaluable. To be evaluable, patients must have undergone at least 4 weeks of treatment, unless definitive disease progression was established after the second or third cycle.

Objective remission and disease progression during therapy were evaluated according to the WHO criteria (11).

Previously irradiated lesions were considered to be unevaluable for response. The occurrence of CNS metastases was not considered to be disease progression.

In contrast to the WHO criteria, duration of response was calculated from Day 1 of remission until the onset of disease progression.

Informed consent was given by all patients in the trial.

Differences between groups of unpaired data in this study were tested for statistical significance by the nonparametic Wilcoxon rank-sum test. $2\alpha = 0.05$ was chosen as the level of significance.

## RESULTS

The trial consisted of 63 patients: 22 with squamous cell carcinoma (WHO I), 25 with adenocarcinoma (WHO III), and 16 with large cell carcinoma (WHO IV). Fifty-three of the patients were previously untreated. Among patients with squamous cell carcinoma, three had previously received DDMP (a folinic acid antagonist), while either the primary tumor or a supraclavicular lymph node had been irradiated in four. One patient with adenocarcinoma had been treated with 5-FU. Two of the patients with large cell carcinoma had received vincristine; one of them was also treated with CCNU, cyclophosphamide, and VP-16-213. Three of the 22 patients with squamous cell carcinoma were unevaluable. Two patients died 7 and 10 days after one dose of vindesine and therapy was discontinued in the third patient after two doses because of severe constipation. The toxic effects subsided and the patient survived for 83 days.

Two patients with adenocarcinoma died from respiratory insufficiency 10 and 11 days after the first dose of vindesine and a third developed abdominal cramps, myalgias, and fever after the initial dose and refused further therapy. The symptoms subsided within 5 days, and the patient survived for 261 days without further chemotherapy.

One patient with large cell carcinoma refused further vindesine after the first dose because of severe constipation and general weakness (survival, 123 days), another did not continue treatment after the initial dose and died after 23 days, and a third died after 7 days.

Data regarding age, sex, and initial performance status of the patients are summarized in table 2.

Biopsies providing the histologic diagnoses were obtained from lungs or mediastinum in 89% of the patients and from scalene nodes in 11%. No specific staging procedures were performed, but dissemination of the disease outside the thoracic cavity and the cervical lymph nodes was confirmed in 49% of the patients.

Two partial remissions lasting 63 and 111 days were observed in patients with squamous cell carcinoma. One complete and five partial remissions were observed in the patients with adenocarcinoma. One of these patients had skin and bone metastases, while the remainder of the responders had disease

TABLE 2.—Patient characteristics

| Histology | No. of patients | No. of— | | No. of evaluable patients | Age in yrs (range) | Average performance status* |
|---|---|---|---|---|---|---|
| | | Males | Females | | | |
| Squamous cell carcinoma | 22 | 17 | 5 | 19 | 61 (52–72) | 1.3 |
| Adenocarcinoma | 25 | 18 | 7 | 22 | 58 (37–71) | 1.2 |
| Large cell carcinoma | 16 | 13 | 3 | 13 | 59 (39–65) | 1.5 |

*Every patient was graded according to a scale of 0–IV (WHO).

localized in the chest. The median duration of response was 196 days (range, 71–450+) and was the shortest in the patient with disseminated disease. Only one patient with large cell carcinoma obtained objective remission, which lasted for 175+ days. Therefore, the response rates were 11% (range, 0–23%; 95% confidence limits) for the squamous cell carcinoma group, 27% (range, 11%–50%) for the adenocarcinoma group, and 8% (range, 0–36%) for the large cell carcinoma group.

With regard to prognostic features, 25% of the responders had disseminated disease, which is significantly less than the 49% in the evaluable patients. Age and performance status were not significantly different in responding and nonresponding patients.

A quantitative evaluation of vindesine toxicity was performed in 54 patients who received more than two doses. Thirteen patients received a cumulative dose greater than or equal to the scheduled dose. Thirty-nine patients tolerated at least three fourths of the planned dose, while the dose was more than halved in only one patient. The 54 patients were treated for 542 cycles. In ten of these cycles the treatment was stopped because of leukopenia, and in four it was stopped because of anemia (ie, concentration of hemoglobin < 6.0 mmols/liter). Two of these episodes of anemia were caused by hemorrhage from a gastric ulcer. Dose reductions were necessary in 36 cycles because of leukopenia and in two cycles because of anemia. Thrombocytopenia (< 100,000 cells/mm$^3$) was not observed. In table 3, the 54 patients are characterized according to blood cell counts observed during the vindesine therapy. No septicemia was observed. Transient elevations of the platelet counts to > 550,000/mm$^3$ were observed in ten patients.

Neurotoxicity necessitated discontinuation of vindesine treatment in 28 cycles and reduction of dose in 121. Paresthesias became dose-limiting in 26% of the 54 patients, weakness in 35%, jaw or abdominal pain in 20%, and constipation in 55%. Dose reductions of 15%–25% were necessary in all five patients receiving vindesine for > 6 months, because of weakness and paresthesias. The median total dose of vindesine received by these five patients was 93 mg/m$^2$ (range, 61–144+).

With regard to the reversibility of neurotoxic effects, 19 patients survived ≥ 8 weeks after discontinuation of the vindesine therapy. Three patients were ineligible because of CNS metastases, poor general condition, and toxicity from chemotherapy including vincristine, respectively. The remaining 16 patients received a median dose of 36 mg/m$^2$ (range, 10–75), and recovered almost completely from the side effects within 8 weeks.

Infusion phlebitis without subcutaneous necrosis was observed in five patients.

Forty-eight of the 63 participating patients were outpatients, so only 315 of 4802 treatment days were spent in a hospital.

## DISCUSSION

This phase II trial suggests that vindesine is active in 27% (11%–50%) of patients with adenocarcinoma of the lung, while the activity in squamous cell carcinoma and large cell carcinoma is only minimal.

The sensitivity of adenocarcinomas to vindesine observed in this trial is consistent with data from four other centers (7–9,12). Six of 29 patients with adenocarcinoma obtained objective remissions at the

TABLE 3.—Hematologic toxicity in 54 patients during vindesine therapy

| Toxicity | No. of patients (%) |
|---|---|
| Hemoglobin (mmols/liter)* | |
| < 7 | 18 (33) |
| < 6 | 7 (13) |
| < 5 | 1 (2) |
| Wbc count (cells/mm$^3$) | |
| < 3000 | 21 (39) |
| ≤ 2000 | 10 (19) |
| ≤ 1000 | 0 (0) |
| Platelet count (cells/mm$^3$) | |
| ≥ 1,000,000 | 2 (4) |
| ≥ 600,000 | 8 (15) |
| ≤ 100,000 | 0 (0) |

*7 mmols/liter = 13.5 g/dl.

Memorial Sloan-Kettering Cancer Center in New York, three of eight in Indianapolis, IN, one of seven in Helsinki, Finland, and two of six in Boston, MA. In these four trials the dose of vindesine was 3 mg/m²/week iv. Compilation of data from these trials and our study showed 18 remissions among 72 patients; ie, a response rate of 25% (range, 16%–37%; 95% confidence limits).

An initial dose of 4 mg/m² led to toxicity that was equivalent or greater than that in other trials (5,9) but not to improved results. This may be explained by dose escalations in most of the studies mentioned.

The observed activity of vindesine has prompted trials of combination regimens. In a phase II trial of cisplatin plus vindesine, Gralla et al (13) observed a response rate of 43% among 87 patients with WHO I, III, and IV lung carcinomas. Preliminary data from a randomized trial (12) have not disclosed any significant difference between cisplatin plus vindesine and vindesine alone. A phase III trial (14) comparing vindesine, cisplatin, and cyclophosphamide with the same three agents plus doxorubicin has shown no significant differences between the response rates of the combinations and the response rate obtained with vindesine alone. Other disappointing results with this three-drug combination have been reported by Mitrou and Fischer (15). Consequently, conclusions regarding the effectiveness of vindesine in combination therapy of pulmonary adenocarcinomas can hardly be drawn.

With regard to the activity of vindesine in the treatment of squamous cell carcinoma, the present data are conflicting. Our negative results are supported by the observations of no response among 18 patients in a phase II trial by Mattson et al (9) and of no response among nine patients in a phase III trial by Hong et al (12). Better responses were seen in three other reported trials. Gralla et al (7) observed three responses among 14 patients, Furnas et al (8) obtained five remissions among 15 patients, and Luedke et al (16) observed six responses among 21 patients.

A compilation of these data showed 16 responses among 96 patients, a response rate of 17% (range, 10%–26%). Most of the patients in these trials were previously untreated, so pretreatment status cannot explain the variations among the studies. Apparently there exists a subpopulation of patients with squamous cell carcinomas sensitive to vindesine. The search for biologic features, including specific histologic findings, characterizing these sensitive tumors should be intensified.

The number of patients with large cell carcinomas has not been sufficient to permit a statistical evaluation of possible activity of vindesine in this cell type in any of the above-mentioned phase II trials (5,8,9,12). Compilation of our data with data from these four studies showed five responses among 27 evaluable patients, with a response rate of 19% (range, 6%–38%). A special methodologic problem is encountered in the histopathologic diagnosis of this cell type because tumors composed of both small cell and large cell elements have a clinical sensitivity to chemotherapy very similar to that of pure small cell carcinomas (17). It is therefore proposed that future trials of lung carcinomas classified as WHO I, III, or IV cell types should direct greater attention to the histologic features.

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# Inhibition of Cardiac Guanylate Cyclase by Doxorubicin and Some of Its Analogs: Possible Relationship to Cardiotoxicity[1,2]

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## SUMMARY

**The anthracycline antibiotic doxorubicin induces a variety of cardiotoxic effects. We have recently demonstrated that this drug also causes a selective inhibition of rat and human cardiac guanylate cyclase activity in vitro. In the present study, we examined the effect of 30 analogs of doxorubicin on cardiac guanylate cyclase activity. Structural modifications of these anthracycline antibiotics were found to alter their effect on rat cardiac guanylate cyclase activity. *N*-Substitutions on the sugar moiety eliminated the inhibitory action observed with the parent compound. Long-chain hydrocarbon substitutions in place of the methylketone side chain had a similar effect. Removal or substitution of the C-4 methoxy group had little or no effect on the ability of these compounds to modify guanylate cyclase activity. Substitutions of the C-9 side chain by a hydrazone derivative resulted in compounds that stimulated the enzyme. All of the anthracenedione derivatives were inhibitory. A comparison of the inhibitory effect of some of these anthracycline derivatives on in vitro cardiac guanylate cyclase activity with their cardiotoxic potency suggests a possible relationship between these two parameters.**

[Cancer Treat Rep 66:311–316, 1982]

The anthracycline antibiotic doxorubicin is an effective agent in the treatment of a variety of solid tumors. However, its clinical use has been limited by the appearance of a variety of dose-dependent cardiotoxic effects which increase greatly in frequency when the maximum cumulative dose is > 550 mg/m² (1–3). The mechanisms of the cardiotoxicity are thus far unexplained.

We have recently demonstrated that doxorubicin causes a selective inhibition of rat and human cardiac guanylate cyclase activity in vitro (4). Additional experiments indicate that a similar inhibition could be demonstrated 24 hours after single-dose in vivo injection of rats (5). In contrast, when the structurally related analog aclacinomycin was tested in the same system, no inhibition of guanylate cyclase activity was observed (6). Since aclacinomycin is much less cardiotoxic than doxorubicin or daunorubicin (41), we considered the possibility that inhibition of cardiac guanylate cyclase may be an indicator of the potential cardiotoxicity of anthracycline antibiotics. In the present study, we examined the effect of > 30 analogs of doxorubicin (fig 1) on the activity of guanylate cyclase from rat hearts in vitro. We hoped to determine the key structural determinants affecting the inhibition of guanylate cyclase activity and, if possible, to correlate the effects on guanylate cyclase with known degrees of cardiotoxicity of the analogs.

## MATERIALS AND METHODS

Doxorubicin, daunorubicin, and all of the analogs tested were gifts from Dr. N. Bachur, Chairman of the Anthracycline Coordinating Group, Division of

[1]Received June 16, 1980; revised July 10, 1981; accepted July 30, 1981.

[2]Supported by Public Health Service grant HL-13715-09 from the National Heart and Lung Institute, National Institutes of Health, Department of Health and Human Services.

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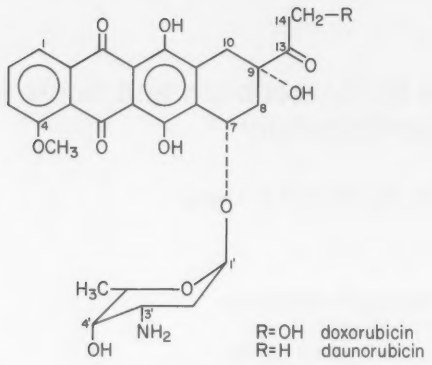


FIGURE 1.—Structure of doxorubicin and daunorubicin.

Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Each compound was dissolved in DMSO or water (depending on its solubility) and was tested at a final concentration of 1 mM. Solutions of these substances were freshly prepared just prior to use in the assay. All controls contained an appropriate DMSO or water mixture.

Guanylate cyclase activity was measured as previously described (4,7). Male Sprague-Dawley rats weighing 175 g were killed by cervical dislocation. Each heart was homogenized in 2.5 ml of cold 30 mM Tris-HCl, pH 7.6, and was centrifuged at 37,000 × *g* in a Sorvall refrigerated centrifuge at 4°C for 10 minutes. The supernatant to which doxorubicin or other antibiotics had been added was assayed at 37°C for 10 minutes for guanylate cyclase activity, using a reaction mixture consisting of 20 mM Tris-HCl (pH 7.5), 5 mM $MnCl_2$, 2.67 mM cyclic GMP, a GTP regenerating system (5 mM creatine phosphate, 11.25 units of creatine phosphokinase), 100 μg of bovine serum albumin, 1 mM 3-iso-butyl-1-methyl-xanthine, and 1.2 mM ($\alpha^{32}$P)-GTP (approximately 5 × $10^5$ cpm; International Chemical and Nuclear Corp, Irvine, CA). The enzyme preparation (supernatant) contained about 50–200 μg of protein in a final volume of 75 μl. The reaction was terminated by the addition of 10 μl of 2 mM EDTA (pH 7.6) containing about 30,000 cpm of $^3$H-cyclic GMP (to estimate recovery in the subsequent steps) and by boiling for 3 minutes. After cooling in an ice bath, the $^{32}$P-cyclic GMP formed was isolated by sequential chromatography of Dowex-50-$H^+$ and alumina (Brinkman Instruments, Westbury, NY) using the modifications of Krishna and Krishnan (7). Eluates from the alumina column were collected directly into scintillation vials containing 15 ml of Bray's solution (8). The eluates were then counted in a Packard Tri-Carb Liquid Scintillation spectrometer. The overall recovery of cyclic GMP after the two-stage chromatographic procedure was 90%–95%. Blank $^{32}$P-counting rates averaged 40–50 cpm. With this assay system, cyclic GMP production was linear with time for at least 20 minutes and with added protein from 50 to 200 μg.

All of the $^{32}$P-containing material was identifiable as cyclic GMP by thin-layer chromatography on PEI cellulose (Brinkman Instruments, NY) using 1 mM LiCl as solvent, and on silicic acid using Chromar 1000 sheets (Mallinckrodt, St. Louis, MO) developed with absolute alcohol and concentrated $NH_4OH$ (5:2 vol/vol) (42).

## RESULTS

Rat heart guanylate cyclase activity was measured in the presence of several anthracycline antibiotics, many of them structural analogs of doxorubicin. Of the 30 compounds tested, ten consistently inhibited, four stimulated, and 16 were weakly inhibitory or stimulatory without being statistically different from the control. Water or DMSO controls had no effect on enzyme activity. Table 1 lists the name and effect on cardiac guanylate cyclase activity of the various compounds tested.

## DISCUSSION

The results obtained in our experiments suggest that a number of structural requirements may exist which favor stimulation or inhibition of guanylate cyclase activity by the anthracycline antibiotics tested. These requirements may be summarized as follows:

1. Effects on guanylate cyclase are unaltered by substitution of the methoxy group by a hydroxyl or even hydrogen.
2. Substitution of the hydroxymethyl ketone side chain of doxorubicin at C-9 by other functional groups had a variable effect depending on the substituent:
   (*a*) Complete removal of the side chains, with only the OH remaining, reduced the inhibitory effect to about one half that of daunorubicin (daunorubicin 9-desacetyl).
   (*b*) Substitution by other short side chains such as the 3-oxime also reduced inhibitory activity to about one half that of the parent compound.
   (*c*) Substitution of a long chain hydrocarbon in place of the methylketone side chain eliminated the inhibitory effect observed with the parent compound, possibly due to

TABLE 1.—Effect of anthracycline antibiotic antitumor agents and anthracenediones on cardiac guanylate cyclase activity*

| NSC No. | Name | % change from control |
|---|---|---|
| | ***Parent compounds and short-chain derivatives*** | |
| 256942 | 4-Epi doxorubicin | −56 |
| 123127 | Doxorubicin | −44 |
| 143491 | Doxorubicin 3-oxime | −24 |
| 263673 | Daunorubicin | −23 |
| 180024 | Carminomycin | −22 |
| 256439 | Demethoxydaunorubicin | −22 |
| 149584 | Doxorubicin-14-nicotinate | −21 |
| 268708 | Daunorubicin-9-desacetyl | −13 |
| | ***Anthracenedione derivatives*** | |
| 301739 | Dihydroxyanthracenedione dihydrochloride | −47 |
| 279836 | Dihydroxyanthracenedione | −24 |
| 196473 | 9,10,Anthracenedione | −20 |
| 299195 | Dihydroxyanthracenedione, diacetate | −18 |
| 287513 | 9,10,Anthracenedione, diacetate | −14 |
| | ***Sugar or long-chain hydrocarbon derivatives*** | |
| 264071 | Doxorubicin, *N*-dodecanoyl | − 8 |
| 261057 | Doxorubicin, 14-stearoyl glycolate | − 4 |
| 169537 | Doxorubicin-DNA | − 2 |
| 261045 | Doxorubicin, *N*,*N*-dimethyl | − 2 |
| 269148 | 7-OMEN | + 2 |
| 246131 | AD 32 | + 3 |
| 258812 | Daunorubicin, *N*,*N*-dimethyl | + 5 |
| 267703 | Quelamycin | + 6 |
| 268242 | Daunorubicin, *N*,*N*-dibenzoyl | +14 |
| 208734 | Aclacinomycin A | +19 |
| | ***Hydrazone derivatives*** | |
| 146496 | Daunorubicin glycoloylhydrazone HCl | + 2 |
| 221265 | Daunorubicin, *m*-nitrobenzoyl hydrazone | + 9 |
| 237672 | Doxorubicin, 3,4,dichlorobenzoyl hydrazone | +19 |
| 164011 | Rubidazone | +25 |
| 219977 | Daunorubicin, *p*-chlorobenzoyl hydrazone | +26 |
| 233853 | Doxorubicin, octanoyl hydrazone | +26 |
| 266210 | Daunorubicin, bis-succinoyl-hydrazone | +36 |

*Results are the mean values of at least 3 experiments expressed as % change from control. Control guanylate cyclase activity = 195 pmols of cyclic GMP produced/mg protein/10 mins. Average coefficient of variability of the individual values ≤ 9%. Enzyme activities different from the control by > 20% were significant ($P < 0.05$). Changes of < 20% from the control were not significantly different as determined by the paired Student's t test.

increased lipid solubility or steric hindrance.Two compounds, doxorubicin 14-stearoylglycolate and AD 32, are examples of this type of structural modification.

(*d*) If the C-9 side chain is replaced by a hydrazone derivative, the compound will stimulate guanylate cyclase. This stimulation may be analogous to the stimulation of guanylate cyclase observed with hydrazine and phenylhydrazine (9,10), compounds which under appropriate conditions may be converted to nitric oxide, an activator of guanylate cyclase (11).

3. Complexes of doxorubicin or daunorubicin with iron or DNA significantly decreased or eliminated the inhibitory effect seen with the parent compounds (doxorubicin-DNA and quelamycin).
4. Substitutions on the $NH_2$ group of daunosamine, or replacement of this amino sugar by any other sugar, eliminated the inhibitory effect observed with the parent compound (doxorubicin, *N*,*N*-dimethyl; AD 32; aclacinomycin A).
5. All of the anthracenedione derivatives which contain no sugar residue were inhibitors of guanylate cyclase activity.

It is evident from the foregoing that the inhibition and stimulation of guanylate cyclase probably occurs by several different mechanisms.

Doxorubicin, daunorubicin, and other anthracyclines which contain quinone groups have been shown by a number of investigators to be involved in the generation of semiquinone free radicals and lipid peroxidation (12–15). Bachur et al (16) have demonstrated that a microsomal NADPH cytochrome P-450 reductase is involved in this reaction. These authors, as well as others, have postulated that the cytotoxic and cardiotoxic effects of these anthracycline antibiotics are related to their ability to enhance the generation of free radicals (13–16). In support of this hypothesis is the observation that scavengers of free radicals (eg, α-tocopherol) reduce both the extent of free radical formation as well as lipid peroxidation and cardiotoxicity (17,18). Several recent publications, however, have questioned the validity of the claim that vitamin E protects against cardiotoxicity (38–40). Free radicals have been shown to be activators of guanylate cyclase (11). However, inhibition of guanylate cyclase by hormones, ions, or free radicals may also be possible, depending on the dose (19–26).

The reason for the apparent correlation between the inhibitory activity observed and the presence of the unsubstituted daunosamine sugar moiety is unclear. The inhibition seen with the anthracenedione compounds which have no sugar residues but are able to form semiquinone free radicals is consistent with the hypothesis that inhibition of guanylate cyclase activity is in some way a result of redox cycling of these structures. At the same time it also suggests that the sugar residue is not an essential feature of the molecule for the inhibitory effect. It may be that the presence of an unsubstituted daunosamine does *not* prevent the generation of free radicals by the quinone structure, while a different sugar, or a substituted daunosamine, does. Alternatively, the anthracyclines may be converted rapidly to the aglycone by the crude enzyme preparation, and it is this conversion which is inhibited by the presence of a substituted daunosamine. The present data do not allow us to answer this question.

Studies of the comparative cardiotoxicity of several of these analogs have been done by many investigators (27–37).

To relate the cardiotoxic effect and the ability of these agents to inhibit guanylate cyclase is difficult. Relative cardiotoxicity is determined by comparing a group of compounds according to the amount needed to produce a fixed effect.

In our experiments, we tested the various analogs at a fixed concentration and compared the variability of the effect. One way to interpret such results is to postulate differences in potency. To test each compound at several concentrations is not feasible due to the limited amounts of material available and the insolubility of some of these substances at high concentrations. At least five of the compounds listed in table 1 (doxorubicin, daunorubicin, 7-OMEN, AD 32, and aclacinomycin) have been tested at various concentrations up to 5 mM (4,6). The dose-response curves obtained indicate that differences in potency are indeed possible and are probably responsible for the differences in inhibitory effect. It is also possible that while some of the compounds tested do inhibit guanylate cyclase at any concentration, others do *not*.

Because of the differences in methodology used, it is not possible to produce a list of doxorubicin analogs in the order of their increasing or decreasing cardiotoxic potency. It is also difficult to relate the differences in the ability of the various analogs to inhibit cardiac guanylate cyclase to the cardiotoxic potency of these same drugs.

The observation that more cardiotoxic analogs such as daunorubicin, or doxorubicin itself are better inhibitors of cardiac guanylate cyclase than the less cardiotoxic analogs [eg, aclacinomycin (41) or 7-OMEN (35)] is interesting, and suggests that additional experimentation may reveal some quantitative or causal relationship between cardiotoxic potency and the ability of anthracycline analogs to inhibit cardiac guanylate cyclase. If such a relationship exists, it does not hold true for the hydrazine derivatives, most of which stimulate guanylate cyclase from the rat heart. These compounds probably alter the activity of this enzyme by a different mechanism from that of the parent compound. The finding that anthracenediones inhibit cardiac guanylate cyclase suggests that inhibition of guanylate cyclase activity may not be an exclusive property of cardiotoxic anthracyclines.

The effects described above were observed at about 1 mM drug concentrations. Such concentrations may appear to be high. Bachur et al (43) have found that a doxorubicin dose of 5 mg/kg produces a cardiac tissue level of 2.6 μg/g of tissue wet weight 8 hours after drug administration. This would be equal to a concentration of about $5 \times 10^{-6}$ M. Tissue concentrations may be higher than this at other times, particularly earlier. Also, tissue levels do not reflect intracellular or subcellular concentration, which may be much higher for a drug with a lypophilic moiety and which is rapidly taken up into the nucleus. Therefore, we see no real problem with describing phenomena that are specific for doxorubicin but which are seen only at high concentrations.

The effects described above are called selective because they were observed in the heart tissue alone; no inhibition was seen in other tissues (4). Other

enzymes such as adenylate cyclase and phosphodiesterase were also tested and were found *not* to be affected by doxorubicin or its analogs (6).

In summary, we have demonstrated several structural requirements for inhibition or stimulation of cardiac guanylate cyclase activity by various anthracycline antibiotics. It should be emphasized that modification of the activity of this enzyme is probably by several mechanisms, and cardiotoxicity is not directly linked to the ability of an agent to inhibit cardiac guanylate cyclase. Additional work is continuing in our laboratory so that we may gain a better understanding both of the mechanisms by which anthracyclines alter cardiac guanylate cyclase activity and of the possible relationship of these phenomena to cardiotoxicity.

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# Comparison of the Effects on Cultured L1210 Leukemia Cells of the Ribosyl, 2′-Deoxyribosyl, and Xylosyl Homologs of Tubercidin and Adenosine Alone or in Combination With 2′-Deoxycoformycin[1,2]

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## SUMMARY

**The biologic effects of a series of sugar-substituted analogs of tubercidin were evaluated and compared with the effects of the homologous series of adenosine analogs in combination with 2′-deoxycoformycin. The greatest cytotoxicity against cultured mouse L1210 leukemia cells was exhibited by tubercidin and by 3′-deoxyadenosine or xylosyladenine in combination with 2′-deoxycoformycin. Somewhat less active were xylotubercidin and the combination of arabinosyladenine (araA) plus 2′-deoxycoformycin. The arabinosyl and 2′- and 3′-deoxyribosyl derivatives of tubercidin were relatively ineffective in their ability to inhibit proliferation of L1210 cells. The major biochemical effects of the most active agents were inhibition of RNA synthesis (3′-deoxyadenosine and xylosyladenine) and depletion of cellular ATP plus general inhibition of macromolecular synthesis (tubercidin). The in vitro activities of xylosyladenine and 3′-deoxyadenosine (in combination with 2′-deoxycoformycin) and xylotubercidin (as a single agent) were greater than or equivalent to that of araA (in combination with 2′-deoxycoformycin).**

[Cancer Treat Rep 66:317–326, 1982]

The use of many structural analogs of adenosine as anticancer agents has been limited by their rapid conversion to biologically inactive products by the enzyme adenosine deaminase (adenosine aminohydrolase, E. C. 3.5.4.4). The recent availability of inhibitors of adenosine deaminase, such as 2′-deoxycoformycin and *erythro*-9(2-hydroxy-3-nonyl)adenine (EHNA), has stimulated a reassessment of the therapeutic activity of certain of the deaminase-sensitive analogs in combination with the deaminase inhibitors. The most well-known example is arabinosyladenine (araA), a potentially useful drug whose activity against cultured cells and transplantable tumors of mice is significantly enhanced by either EHNA or 2′-deoxycoformycin (2). Preclinical toxicology studies with araA and its more soluble derivative araAMP in combination with 2′-deoxycoformycin are currently under way in preparation for clinical trials of these two-drug combinations in cancer patients.

This study was undertaken to evaluate the biologic activity of a series of sugar-modified analogs of tubercidin (3–5) and to compare their effects with those of the homologous compounds in combination with 2′-deoxycoformycin. Included were several deaminase-sensitive analogs of adenosine that are cytotoxic against cultured cells when protected from deamination by addition of deaminase inhibitor to the culture medium (6,7). Although tubercidin is a substrate for many enzymes concerned with processes involving adenosine and its phosphorylated intermediates, it is not subject to deamination or phosphorolysis, the two major degradative pathways for adenosine and many other

[1]Received Nov 7, 1980; revised June 4, 1981; accepted June 23, 1981.

[2]Supported by the National Cancer Institute of Canada. Portions of this work were described in a preliminary report (*see* ref 1).

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biologically active analogs of adenosine. A comprehensive review (8), recently updated (9), outlines the chemistry of tubercidin and results of biochemical, biologic, and clinical studies. Many different biochemical effects have been attributed to tubercidin, including interference with energy metabolism and inhibition of de novo synthesis of purines, processing of rRNA, and synthesis of nucleic acids and proteins. The serious side effects of tubercidin (10,11), which were considered intolerable in clinical studies (12,13), are undoubtedly due to its many different modes of action.

The chemical synthesis of modified sugar analogs of tubercidin was undertaken to obtain agents that retain resistance to deamination and phosphorolysis but have fewer biochemical effects than tubercidin. The synthetic procedures are reported elsewhere (3–5). By analogy with the homologous sugar-modified analogs of adenosine, the xylosyl, arabinosyl, and 2′- and 3′-deoxyribosyl derivatives of tubercidin were candidates for more specific effects on RNA or DNA synthesis or function, either through inhibition of, or participation in, polymerase-dependent reactions. Although none of the tubercidin derivatives was as active against cultured L1210 cells as the parent compound, the xylosyl and arabinosyl derivatives were sufficiently cytotoxic to warrant detailed study.

## MATERIALS AND METHODS

The origin of the cultured line of mouse L1210 leukemia cells, designated L1210/C2, was described previously (14). The L1210/C2 cell line is a clonal isolate that exhibits in vivo growth kinetics and tumorigenic properties comparable to those of the parental transplantable tumor. Suspension cultures of L1210/C2 cells were routinely maintained at 37°C without antibiotics under conditions of logarithmic growth in Fischer's medium supplemented with 10% horse serum. Cultures were initiated from mycoplasma-free stocks stored in liquid nitrogen and were carried for 25–30 subculture generations and then discarded. For experimental cultures, the growth medium also contained penicillin (100 units/ml), streptomycin (100 μg/ml), and in some cases gentamicin (50 μg/ml). Cell culture reagents were obtained from Grand Island Biological Co, Burlington, Ontario, Canada.

Drug effects on the proliferation of L1210/C2 cells were determined as follows. Drug exposures were initiated by combining equal portions of logarithmically proliferating suspension cultures ($2 \times 10^5$ cells/ml) with fresh growth medium containing additive(s) at twice the concentration to be tested; at least two cultures were prepared for each condition to be studied. Cultures were incubated at 37°C in loosely capped bottles in 5% $CO_2$ in air, and cell numbers were determined at 24-hr intervals using an electronic particle counter. In some experiments, cell volume determinations were also performed using an electronic counter with a multichannel particle analyzer and an X-Y recorder (Channelyzer II; Coulter Electronics Inc, Hialeah, FL).

A soft agar cloning procedure (14) was used to determine drug effects on cell viability. Suspension cultures were exposed to drugs for 24-hr intervals as described above. Cells were collected by centrifugation, washed once, and resuspended in cloning medium (Fischer's medium supplemented with 50 μg/ml of gentamicin, 10% horse serum, and 10% conditioned medium, which was obtained by centrifugation of 48-hr cultures followed by filtration through 0.22-μ nitrocellulose filters). Cell suspensions prepared in cloning medium containing 0.13% Noble agar (5 ml/culture tube) were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ in air; after 2 weeks, the agar was poured into Petri dishes for counting with a dissecting microscope at low magnification.

Drug effects on tumorigenicity were assayed as described elsewhere (14). L1210/C2 cells were exposed to drug under cell culture conditions as outlined above and then implanted ip in 0.25-ml volumes of drug-free growth medium into groups of six or 12 female mice. The mice used, C57BL × DBA/2 (hereinafter called $BDF_1$), were obtained from the Health Sciences Animal Breeding Unit, University of Alberta, Edmonton, Alberta, Canada.

Drug effects on macromolecular synthesis were assessed by determination of rates of incorporation of the appropriate $^3H$-labeled precursor into acid-insoluble fractions using a filtration procedure. Suspension cultures (about $10^5$ cells/ml) were exposed to drugs for 2-hr intervals as described above after which 15-ml portions were removed, mixed with 100 μl of 0.9% NaCl solution containing $^3H$-labeled precursor, gassed with 10% $CO_2$ in air, and incubated at 37°C with gentle shaking. At timed intervals thereafter, 1-ml portions of incubation mixtures were added to 5 ml of cold 0.9% NaCl solution, and cells were rapidly collected on 1.2-μ nitrocellulose filters and extracted several times with 5 ml of cold 5% trichloroacetic acid. After being washed with cold water, the filters were air-dried and combusted in a Packard Model 306 Sample Oxidizer for determination of $^3H$ content by liquid scintillation counting.

The effects of drug treatment on concentrations of perchloric acid-soluble purine and pyrimidine ribonucleotides were determined spectrophotomet-

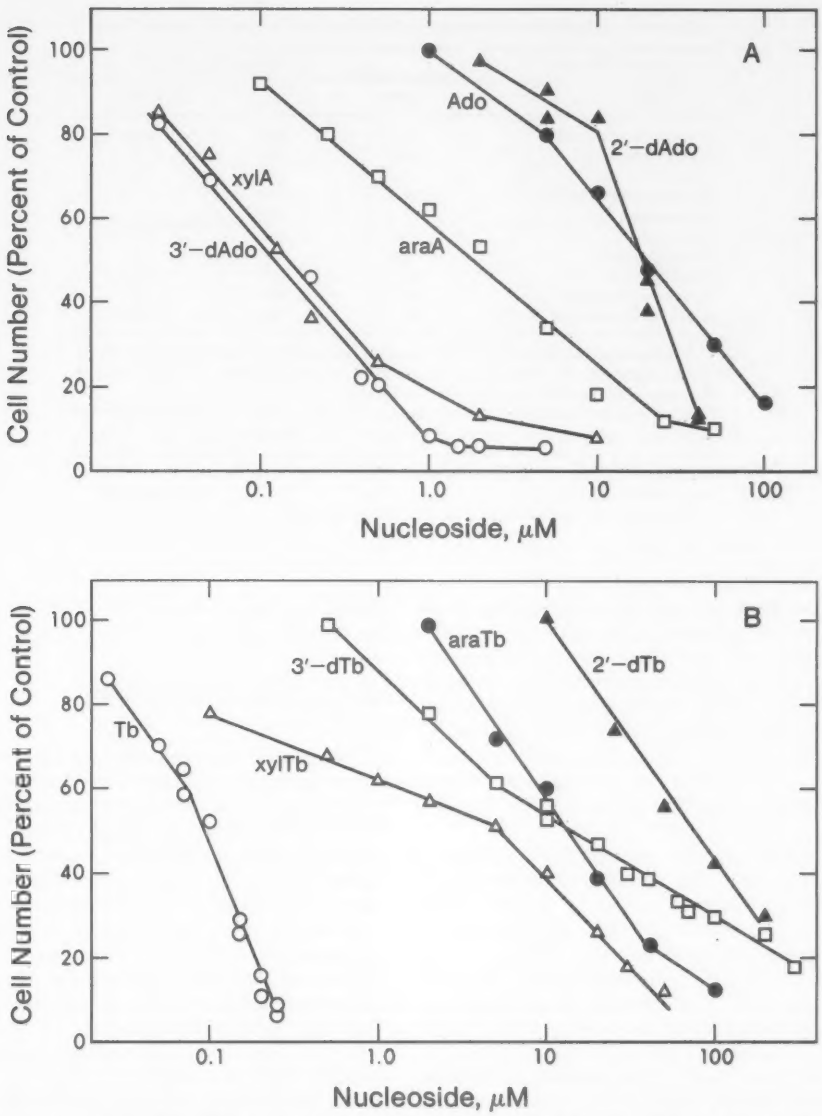


FIGURE 1.—Concentration-effect relationships for analog inhibition of L1210/C2 cell proliferation. Duplicate 20-ml cultures, initiated at $10^5$ cells/ml, were incubated at 37°C in the absence (controls) or presence of each drug at the concentrations indicated. Samples were removed at 24-hr intervals for determination of cell numbers as described in the Materials and Methods section. Plotted are the values (expressed as % of untreated control cultures) for cell numbers after 48-hr exposures. Adenosine and its analogs (group 1) were tested in the presence of 1 μM 2′-deoxycoformycin (panel A) and tubercidin and its analogs (group 2) were tested in the absence of 2′-deoxycoformycin (panel B). IC50 values (drug concentrations that inhibited an increase in cell numbers by approximately 50% after a 48-hr exposure) are listed in table 1.

rically after their separation by high-pressure liquid chromatography using a Reeve Angel Partisil-10AX column (25 cm × 4.6 mm; Mandel Scientific, Montreal, Quebec, Canada) and a linear gradient of potassium phosphate and potassium chloride, pH 4.5, as described in detail elsewhere (15).

Xylotubercidin, aratubercidin, and deoxytubercidin were synthesized as described previously (3–

TABLE 1.—Effect of 48-hr exposure to drug on the volume of L1210/C2 cells

| Drug | Concentration (μM)* | Mean cell volume† | |
|---|---|---|---|
| | | $\mu^3$ | T/C |
| 3′-Deoxyadenosine‡ | 0.1 | 390 | 1.2 |
| Xylosyladenine‡ | 0.2 | 445 | 1.3 |
| AraA‡ | 2 | 602 | 1.8 |
| 2′-Deoxyadenosine‡ | 20 | 402 | 1.4 |
| Adenosine‡ | 20 | 478 | 1.7 |
| Tubercidin | 0.1 | 485 | 1.3 |
| Xylotubercidin | 5.0 | 587 | 1.6 |
| Aratubercidin | 15 | 616 | 1.7 |
| 3′-Deoxytubercidin | 20 | 457 | 1.1 |
| 2′-Deoxytubercidin | 78 | 334 | 0.9 |

*Concentrations used were IC50 values obtained from the experiments of fig 1 and represent the drug concentration that inhibited increase in cell numbers by approximately 50% after a 48-hr exposure.

†Cell volume distributions were determined after 48-hr exposures; presented are mean cell volumes, computed as described in the Materials and Methods section, and the ratio of mean cell volume of treated (T) cultures to that of untreated control (C) cultures (control volumes in different experiments ranged from 290 to 410 $\mu^3$).

‡Tested in the presence of 1 μM 2′-deoxycoformycin.

5). 2′-Deoxycoformycin was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, and nitrobenzylthioinosine was provided by Dr. A. R. P. Paterson of the McEachern Laboratory. Other nucleosides and nucleoside analogs were obtained from commercial sources. [5-$^3$H]Uridine, L-[4,5-$^3$H]leucine, and [methyl-$^3$H]thymidine were purchased from Amersham/Searle, Arlington Heights, IL, and from Moravek Biochemicals, City of Industry, CA.

Xylotubercidin (3) was purified from a trace of accompanying tubercidin (about 2%) by treatment with sodium periodate followed by sodium borohydride (16). Separation of the oxidized-reduced tubercidin from xylotubercidin was effected easily using Dowex 1-X2 ($OH^-$) chromatography. Product recovery was high as illustrated in the following control experiment. A mixture of 3 mg of tubercidin and 1.3 mg of xylotubercidin was stirred with 2 ml of 0.01 M aqueous $NaIO_4$ solution for 1 hr at room temperature. $NaBH_4$ (about 2 mg) was added and stirring was continued for 30 minutes. The reaction mixture was applied to a column (2 × 8 cm) of Dowex 1-X2 ($OH^-$) resin and 16-ml fractions were collected. Stepwise elution (using 200-ml portions of pure water up to 60% methanol/water in increasing increments of 10% methanol) gave 3 mg of ox-red tubercidin in fractions 14–22 and 1.2 mg of xylotubercidin in fractions 66–75.

Crude synthetic xylotubercidin (3) was treated analogously with excess sodium periodate and sodium borohydride, followed by column chromatography on Dowex 1-X2 ($OH^-$) and recrystallization to give the pure nucleoside.

## RESULTS AND DISCUSSION

The cytostatic properties of the deaminase-sensitive (group 1) and deaminase-insensitive (group 2) analogs were compared by assessing their ability to inhibit proliferation of cultured L1210 leukemia cells during prolonged periods of continuous drug exposure (fig 1). Each agent was evaluated in the presence and absence of 2′-deoxycoformycin at a concentration previously shown (14,17) to inhibit deamination of araA or adenosine over the time periods used for these experiments. As expected, the cytostatic activity of the deaminase-sensitive analogs was greatly increased by the presence of 2′-deoxycoformycin in culture fluids, whereas those of the deaminase-insensitive analogs were not affected (data not shown). In subsequent experiments, we have compared the effects of group 1 analogs in combination with 2′-deoxycoformycin with those of group 2 analogs used as single agents.

The most potent antiproliferative agents among the ten compounds tested were 3′-deoxyadenosine and xylosyladenine in group 1 and tubercidin in group 2. These agents exhibited relatively steep dose-response curves and IC50 values of 0.1–0.15 μM. AraA and xylotubercidin were somewhat less effective, with IC50 values of 2 and 5 μM, respectively, and relatively broad dose-response curves. The least ef-

TABLE 2.—Effect of drug exposure on viability*

| Analog | Concentration (μM) | Cloning efficiency† | |
|---|---|---|---|
| | | % | T/C |
| Xylosyladenine‡ | 0 | 80 | 1.0 |
| | 0.4 | 17 | 0.21 |
| | 1.0 | 2 | 0.03 |
| | 5.0 | 0.002 | 0.00003 |
| 3′-Deoxyadenosine‡ | 0 | 84 | 1.0 |
| | 0.4 | 41 | 0.49 |
| | 1.0 | 12 | 0.14 |
| | 5.0 | 4 | 0.05 |
| AraA‡ | 0 | 84 | 1.0 |
| | 1 | 52 | 0.62 |
| | 5 | 6.3 | 0.08 |
| | 10 | 0.7 | 0.01 |
| Tubercidin | 0 | 68 | 1.0 |
| | 0.05 | 54 | 0.79 |
| | 0.075 | 27 | 0.40 |
| | 0.15 | 2.5 | 0.04 |
| Xylotubercidin | 0 | 85 | 1.0 |
| | 20 | 21 | 0.25 |
| | 50 | 4 | 0.05 |
| | 100 | 0.13 | 0.001 |
| Aratubercidin | 0 | 66 | 1.0 |
| | 10 | 48 | 0.77 |
| | 20 | 27 | 0.41 |
| | 50 | 6 | 0.09 |

*Logarithmically proliferating L1210/C2 cultures ($10^5$ cells/ml) were exposed to various concentrations of analog at 37°C for 24 hrs and then assayed for viability by the soft agar cloning procedure described in the Materials and Methods section.

†Sets of replicate tubes (5/condition) were inoculated with 100–10,000 cells/tube, depending on the conditions of drug exposure. Presented are (*a*) values for the average No. of colonies/tube (as % of cells inoculated/tube) determined from those sets of tubes which, at the time of counting, contained 20–100 colonies/tube and (*b*) the ratio of cloning efficiencies of treated cells (T) to those of untreated control cells (C).

‡Tested in the presence of 1 μM 2′-deoxycoformycin.

TABLE 3.—Effect of NBMPR on antiproliferative activity*

| Analog | IC50 (μM) | | |
|---|---|---|---|
| | −NBMPR | +NBMPR | +/− |
| 3′-Deoxyadenosine† | 0.1 | 0.2 | 2.0 |
| Xylosyladenine† | 0.2 | 0.8 | 4.0 |
| AraA† | 2‡ | 2.5‡ | 1.3 |
| 2′-Deoxyadenosine† | 19 | 10 | 0.5 |
| Tubercidin | 0.1 | 0.3 | 3.0 |
| Xylotubercidin | 6 | 11 | 1.8 |
| Aratubercidin | 17 | 20 | 1.2 |

*Experiments were conducted as described in fig 1, except that L1210/C2 cells were also exposed to various concentrations of each analog in the presence of 5 μM NBMPR. Approximate IC50 values were determined from cell numbers obtained after 48-hr exposures to drug.

†Tested in the presence of 1 μM 2′-deoxycoformycin.

‡A similar result was reported previously (17).

fective agents were the 2′- and 3′-deoxyribosyl derivatives of tubercidin.

Drug effects on cell size during inhibition of proliferation were evaluated by comparing cell volumes after exposure of cultures for 48 hrs to each agent at its IC50 value (table 1). Substantial increases in cell size were seen with araA, adenosine, xylotubercidin, and aratubercidin; moderate increases were observed with xylosyladenine, 2′-deoxyadenosine, and tubercidin; and little if any change was seen with 3′-deoxyadenosine, 3′-deoxytubercidin, and 2′-deoxytubercidin.

The ability of the most active agents to kill cells was determined by assay of colony formation in soft agar after 24-hr exposures of L1210 cultures to a range of growth-inhibitory concentrations (table 2). Although xylosyladenine and 3′-deoxyadenosine were equally effective in inhibiting proliferation, xylosyladenine was much more effective in killing cells than was 3′-deoxyadenosine (compare, for example, the effects of both agents when tested at 1 and 5 μM), and both were more cytotoxic than was araA. The greatest cytotoxicity among group 2 agents was seen with tubercidin.

The effects of tubercidin and two of its analogs on tumorigenicity of L1210 cells were determined in experiments that assessed the ability of cells, after drug exposure under cell culture conditions, to give rise to ascites tumors when implanted ip into mice. Results from the experiments with tubercidin and aratubercidin are summarized in figure 2. Similar experiments (not shown) were conducted with xylotubercidin. The relative effects on viability in vivo (ie, tumorigenicity) were like those on viability in vitro, with tubercidin exhibiting the greatest activity. Tumorigenicity was reduced by greater than five orders of magnitude after a 2-hr exposure to 1 μM tubercidin, whereas 24-hr exposures to 98 μM aratubercidin or 100 μM xylotubercidin (not shown) reduced tumorigenicity by 90%–99%. We

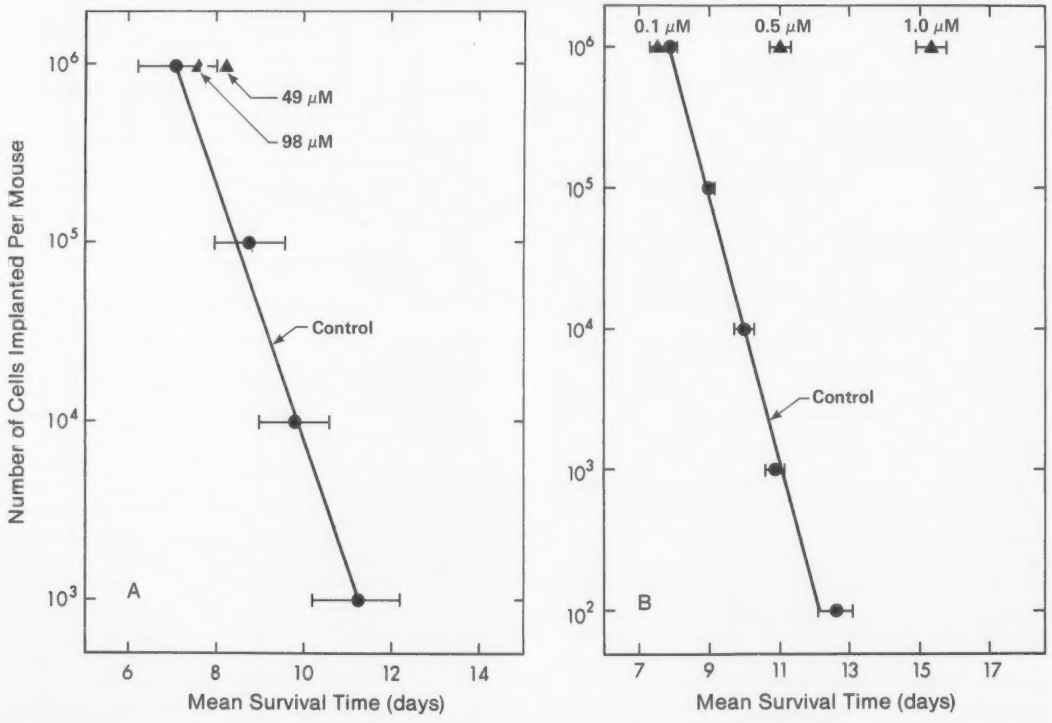


FIGURE 2.—Effects of in vitro exposure to A) aratubercidin or B) tubercidin on tumorigenicity of L1210/C2 cells. L1210/C2 cells in suspension cultures were incubated at 37°C, as described in the Materials and Methods section, in growth medium containing aratubercidin (24-hr incubation) or tubercidin (2-hr incubation) at the concentrations indicated. In each experiment, cells were also incubated in the absence of additives (controls) and subjected to the same manipulations as drug-treated cells. For assay of tumorigenicity, cells were collected by centrifugation, washed and resuspended in drug-free media, and implanted ip into groups of 6 and 12 $BDF_1$ mice.

TABLE 4.—Effect of 2-hr exposure to drug on macromolecular synthesis by L1210/C2 cells*

| Drug | Concentration (μM) | Incorporation (% of control) of— | | |
|---|---|---|---|---|
| | | $^3$H-dThd | $^3$H-Urd | $^3$H-Leu |
| 3′-Deoxyadenosine† | 0.15 | 98 | 74 | 93 |
| | 0.4 | 100 | 67 | 87 |
| Xylosyladenine† | 0.15 | 100 | 84 | — |
| | 0.4 | 91 | 70 | 95 |
| AraA† | 2 | 88 | 101 | 108 |
| | 10 | 21 | 77 | 99 |
| 2′-Deoxyadenosine† | 20 | 57 | 77 | 104 |
| | 50 | 11 | 59 | 86 |
| Adenosine† | 19 | 93 | 92 | 104 |
| | 100 | 55 | 51 | 85 |
| Tubercidin | 0.1 | 86 | 72 | 62 |
| | 0.25 | 72 | 50 | 41 |
| Xylotubercidin | 6 | 70 | 100 | 96 |
| | 20 | 45 | 90 | 93 |
| Aratubercidin | 15 | 50 | 100 | 80 |
| | 98 | 10 | 100 | 70 |
| 3′-Deoxytubercidin | 18 | 83 | 75 | 84 |
| | 50 | 64 | 57 | 78 |
| 2′-Deoxytubercidin‡ | — | — | — | — |
| | 189 | 20 | 50 | 100 |

*Logarithmically proliferating L1210/C2 cells ($10^5$ cells/ml) were incubated at 37°C in the presence or absence of drugs for 2 hrs; unless otherwise noted, the 2 concentrations used for each drug were those that inhibited proliferation by 50% and by 80%–90% in the experiments of fig 1 and table 1. Incorporation of the appropriate $^3$H-labeled precursor into acid-insoluble material was then determined as described in the Materials and Methods section and is expressed for drug-treated cultures as % of incorporation obtained in untreated control cultures.

†Cultures were incubated with 2′-deoxycoformycin (1 μM) for 15 minutes prior to addition of drug.

‡Because of limited availability of drug, experiments were only conducted at the concentration that inhibited proliferation by about 90% after a 48-hr exposure.

have previously reported results of similar studies with araA, in which significant reductions in tumorigenicity were only observed after prolonged exposures (24–72 hrs) to growth-inhibitory levels (10 μM) of araA (14,17).

Cellular uptake has been shown to be an important determinant of the biologic activity of tubercidin and other cytotoxic nucleoside analogs (18). The importance of entry of drug via mediated transport for expression of cytostatic and cytotoxic activity was evaluated by the nitrobenzylthioinosine (NBMPR) protection assay described previously (19). L1210 cells were exposed to group 1 and 2 derivatives in the presence and absence of the potent inhibitor of nucleoside transport NBMPR, and IC50 values for inhibition of proliferation were determined (table 3). Significant protection was observed only for 3′-deoxyadenosine, xylosyladenine, tubercidin, and xylotubercidin. No protective effects were seen with araA or aratubercidin, and the activity of 2′-deoxyadenosine was enhanced twofold by NBMPR. These results indicate a requirement for a functional transporter for activity of 3′-deoxyadenosine, xylosyladenine, tubercidin, and xylotubercidin. AraA and aratubercidin were as active in the presence of the transport inhibitor as in its absence, and the toxicity of 2′-deoxyadenosine was enhanced by NBMPR. Since araA is a substrate for the NBMPR-sensitive transport of L1210 cells,[4] we suspect that cellular uptake of araA by other means (presumably passive diffusion) is sufficient to generate cytotoxic levels of intracellular araA metabolites.

Since the adenosine analogs of group 1 are known to have varied effects on macromolecular synthesis (6,8,9), a comparison was made under standardized

[4]We do not have an explanation for enhancement by NBMPR of 2′-deoxyadenosine activity. Recent experiments using an oil-centrifugation method that allows measurement of initial transport rates (18) have indicated that araA is a substrate for the NBMPR-sensitive transporter of L1210/C2 cells (C. E. Cass, unpublished results).

TABLE 5.—Effects of tubercidin and its derivatives on acid-soluble ribonucleotide levels*

| Drug | Exposure | | % of control values† for— | | | |
|---|---|---|---|---|---|---|
| | μM | Hrs | CTP | UTP | ATP | GTP |
| Tubercidin | 0.5 | 2 | 118 | 99 | 76 | 93 |
| | 1.0 | 2 | 117 | 96 | 60 | 119 |
| | 0.1 | 24 | 72 | 69 | 74 | 76 |
| | 0.5 | 24 | 0 | 0 | 0 | 0 |
| | 1.0 | 24 | 0 | 0 | 0 | 0 |
| Xylotubercidin | 6 | 2 | 97 | 101 | 99 | 97 |
| | 50 | 2 | 116 | 103 | 103 | 106 |
| | 100 | 2 | 179 | 133 | 123 | 142 |
| | 6 | 24 | 95 | 93 | 103 | 118 |
| | 50 | 24 | 98 | 59 | 83 | 135 |
| | 100 | 24 | 38 | 34 | 35 | 40 |
| Aratubercidin‡ | 200 | 2 | 103 | 92 | 103 | 137 |

*Logarithmically proliferating L1210/C2 cultures (1–2 × $10^5$ cells/ml) were exposed to drug at the concentrations indicated at 37°C for 2 or 24 hrs. Cells were collected by centrifugation at 4°C and perchloric acid extracts were prepared for analysis of ribonucleotides by high-pressure liquid chromatography as described in the Materials and Methods section. The effects of each drug were determined in separate experiments.

†Ranges of control values (nmols/$10^9$ cells) in these experiments were: CTP, 195–338; UTP, 759–1207; ATP, 2340–3000; and GTP, 443–781.

‡Tested at only 1 dose/exposure combination because of limited availability of drug.

conditions of the ability of group 1 and 2 analogs to inhibit DNA, RNA, and protein synthesis (table 4). Cultures were exposed to drug at growth-inhibitory concentrations (IC50, IC90) for 2-hr periods before assay of incorporation of the appropriate radioactive precursor into acid-insoluble material. Although most of the analogs had multiple inhibitory effects, only tubercidin was inhibitory against all three processes at its IC50 concentration. Several of the analogs (3′-deoxyadenosine, xylosyladenine, xylotubercidin, araA, and aratubercidin) appeared to inhibit only RNA or DNA synthesis at IC50 concentrations, with multiple effects occurring only at the higher growth-inhibitory concentration, indicating greater specificity of drug metabolites for inhibition of nucleic acid synthesis than of protein synthesis.

Comparison of the effects of xylotubercidin and xylosyladenine on macromolecular synthesis suggests that the underlying biochemical mechanisms of these agents may be different. Xylotubercidin inhibited DNA synthesis, whereas xylosyladenine (plus 2′-deoxycoformycin) primarily inhibited RNA synthesis. The biochemical effects of exposure of cultured cells to xylosyladenine in combination with 2′-deoxycoformycin have recently been reviewed (6). In contrast, the mechanism of action of aratubercidin is probably similar to that of araA (plus 2′-deoxycoformycin). Exposure of cells to aratubercidin produced a syndrome of reduced DNA synthesis and increased cell volume similar to that seen in araA-treated cells. The inhibition of DNA synthesis by araATP is well-established, and results of biochemical, biologic, and clinical studies with this agent have recently been reviewed (2).

Studies with other cell types have shown that tubercidin, after phosphorylation, interferes with a variety of processes dependent on cellular ATP (8,9,20). The effects of tubercidin and its two most active derivatives on cellular levels of ribonucleotides were compared under conditions of partial and complete inhibition of growth. Analysis of perchlorate extracts prepared from drug-treated cells was performed using a high-pressure liquid chromatographic system standardized for detection of purine and pyrimidine ribonucleotides (table 5). The analysis of 2-hr exposures to drug provided an indication of early effects of drug exposure on ribonucleotide levels. These were apparent only in cultures treated with tubercidin (decreased ATP and GTP) and high concentrations of xylotubercidin (increased CTP, UTP, GTP, and ATP). An additional early effect of exposure to tubercidin appeared to be substantial phosphorylation of tubercidin, evident by the presence of an extra peak in the triphosphate region of chromatographs. The size of the extra peak was positively correlated with the concentration of tubercidin and the length of exposure of cultures to tubercidin. Short exposures to aratubercidin or to partially growth-inhibitory concentrations of xylotubercidin were without effect on ribonucleotide levels, and no extra peaks were present.

Analysis of extracts of cells exposed for 24 hrs provided evidence of what were probably secondary effects of drug treatment on ribonucleotide levels. Tubercidin-treated cells either had no detectable ribonucleotides or they were greatly reduced, whereas only cells treated with the highest concentration of xylotubercidin exhibited significant changes in ribonucleotide levels (reduced CTP, UTP, GTP, and ATP).

The biologic studies indicate that further evaluation of the xylosyl and 3′-deoxyribosyl derivatives of adenosine in combination with 2′-deoxycoformycin would be worthwhile. Systematic assessment of antitumor activity and of in vivo toxicity is needed for these agents, which in contrast to araA, appear to be cytotoxic because of their effects on RNA, rather than DNA, synthesis. Although xylosyladenine and 3′-deoxyadenosine are inactive as single agents, they both exhibited significant antitumor activity when used in combination with 2′-deoxycoformycin in the treatment of mice bearing the P388 leukemia (21,22). Only one of the tubercidin derivatives, xylotubercidin, exhibited sufficient in vitro activity to warrant in vivo screening. Although preliminary tests in which xylotubercidin was administered daily for 5 days to mice bearing the ascites L1210 leukemia have provided evidence of marginal antitumor activity (C. E. Cass, M. Selner, W. H. Muhs, et al, unpublished results), other schedules of administration may be more effective.

A final note of caution should be mentioned regarding biologic studies on modified compounds derived from cytotoxic antibiotics. Contamination of a less toxic semisynthetic compound with trace quantities of the parent antibiotic can lead to spurious biologic conlusions. Treatment of the modified tubercidin nucleosides with periodate/sodium borohydride followed by chromatography and re-evaluation in cell culture was employed as a control in this study.[5] It was found that synthetic samples of xylotubercidin (3), which comigrate with tubercidin on Dowex 1-X2 ($OH^-$) columns, contained trace quantities of the parent antibiotic (about 2%). This is sufficient to produce spurious biologic results if not removed as described in the Materials and Methods section. The other modified tubercidin compounds were not affected by this treatment.

[5]Ribonucleosides are oxidized rapidly and quantitatively by sodium periodate to give the sugar dialdehyde derivative (16). Subsequent reduction with sodium borohydride gives a triol (oxidized-reduced) compound (16). In contrast, 2′-deoxy and 4′-deoxy nucleosides that lack the vicinal diol unit are not attacked by periodate. The *trans* xylo and arabino diastereomers of the *cis* ribo diols are attacked very slowly (however, significant loss of product can occur over extended time periods).

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# Isolation and Properties of Chinese Hamster Lung Cells Resistant to Ellipticine Derivatives[1,2]

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## SUMMARY

**Chinese hamster lung cells resistant to 9-OH-ellipticine (9-OH-E) were selected in vitro by adding stepwise increasing drug concentrations to the cell growth medium. This selection procedure resulted in the isolation of two sublines, about tenfold and 12-fold resistant to 9-OH-E. This level of resistance did not increase even after about 30 months of drug exposure. Cytogenetic studies revealed that the resistant cells carry several discrete karyotype modifications, the most striking being the absence of one No. 5 chromosome and the presence of a marker chromosome characterized by peculiar G-banding. Development of 9-OH-E resistance was also associated with some changes in cell properties such as modifications of morphology and growth parameters, lower oncogenic potential, and cross-resistance to a variety of antitumor agents. Although these results suggest that the resistance is associated with a modification of cell membrane properties, drug uptake studies did not show any significant difference between the parental sensitive cells and the resistant sublines.**

[Cancer Treat Rep 66:327–338, 1982]

Ellipticine is a plant alkaloid, derived from pyridocarbazole, which has been shown to have antitumor activity against several experimental tumors (1,2). A natural derivative, 9-$CH_3O$-ellipticine, has been reported to produce remissions in patients with myeloblastic leukemia (3). Both drugs have been shown to bind to DNA through intercalation between adjacent base pairs (4,5). On the assumption that the DNA binding might be related to the cytotoxicity of these drugs, a number of new compounds capable of intercalating into DNA with a high affinity were selected and, among them, several new antitumor agents were obtained (6–8). 9-OH-ellipticine (9-OH-E) elicits high antitumor activity in L1210 mouse leukemia (7,9); 2-*N*-$CH_3$-9-OH-ellipticinium (NMHE) is active against some human tumors and has been submitted to a phase II clinical trial (10).

A number of studies dealing with the mechanism of action of these drugs have been presented. Ellipticine kills cells in all phases of the cell cycle, although cells in M and G1 have been reported to be more sensitive to the drug than cells in S and G2 (11). Ellipticine, as well as some of its derivatives, inhibits DNA, RNA, and protein synthesis (11,12) and interferes with such biologic activities as processing of ribosomal precursor RNA (13) or methylation of RNA (14). Ellipticine has also been shown to induce protein-associated DNA single-strand breaks in vivo (15). Although a drug-nucleic acid interaction seems to be essential to the action of

[1]Received Jan 28, 1981; revised June 24, 1981; accepted July 6, 1981.

[2]Supported by the Centre National de la Recherche Scientifique (L. A. 147), the Institut National de la Santé et de la Recherche Médicale (U 140), the Délégation à la Recherche Scientifique et Technique (DGRST) (78.7.2649), and the Commissariat à l'Energie Atomique. J.-Y. Charcosset was a recipient of a DGRST Fellowship (Membranes biologiques).

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[5]We thank Drs. G. Barski, J. Belehradek, and their collaborators for assistance and advice. We are also indebted to Dr. J. B. Le Pecq for helpful discussions and to Charlotte Delaporte for excellent technical assistance.

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these drugs, the precise mechanism leading to cell death and the nature of the ultimate intracellular target remain unknown.

As already indicated, these antitumor agents were selected on the basis of their DNA affinity. However, this property alone does not account for the antitumor activity. For example, 9-$NH_2$-ellipticine and 9-OH-E have about the same DNA affinities, yet the former is much less active than the latter against L1210 mouse leukemia (16). This indicates that, in addition to DNA affinity, the pharmacologic activity of the ellipticine drugs depends on one or several other structural properties.

To study the mechanism of action and the specificity of these drugs, Chinese hamster lung cells resistant to 9-OH-E were selected in vitro. The derivative 9-OH-E was used because when we started the selection, this molecule had the highest antitumor activity in the drug series. In addition, it was known that, in vivo, 9-OH-E was the major product of ellipticine metabolism (17). This paper describes the isolation and some properties of two Chinese hamster lung cell sublines with different levels of resistance to 9-OH-E. Our results show that, in parallel to the development of resistance to 9-OH-E, the resistant cells undergo several changes: modifications of the morphology and growth parameters, a lower oncogenic potential, and cross-resistance to a variety of antitumor agents. However, studies on the intracellular uptake of a radiolabeled derivative of 9-OH-E failed to show any significant difference between the sensitive and resistant cells. Finally, cytogenetic analysis of the resistant sublines revealed discrete modifications of the karyotype, which remained in the diploid range. The most striking modifications were the loss of one No. 5 chromosome and the presence of a large metacentric chromosome characterized by peculiar G-banding. The significance of these results is discussed.

## MATERIALS AND METHODS

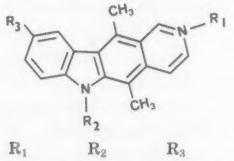
### Cells and Culture Medium

The parental line DC-3F and the dactinomycin-resistant subline DC-3F/AD X, as previously described (18), were supplied by Dr. G. Barski. These Chinese hamster lung cells were grown as monolayer cultures on plastic. Eagle's minimal essential medium (MEM), supplemented with 7% fetal calf serum, penicillin (100 IU/ml), and streptomycin (50 μg/ml), was used throughout.

### Drugs

The ellipticine derivatives used in this work (table 1) were provided by Drs. Nguyen Dat-Xuong and E. Lescot. Compounds A and B (in table 1) are derivatives of 7*H*-pyridocarbazole, which is an ellipticine structural analog. Compound A is the *N*-2-ethylpiperidino-10-methoxy-7*H*-pyridocarbazolium, and compound B is a dimer molecule consisting of two compounds A, linked by the ethylpiperidino groups (19). These molecules were supplied by Dr. B. P. Roques.

TABLE 1.—Structure and cytotoxicity of some ellipticine derivatives on the parental DC-3F line and the DC-3F/9-OH-E 0.3 subline




| Drugs | $R_1$ | $R_2$ | $R_3$ | ED50 for DC-3F (M)* | ED50 ratio (DC-3F/9-OH-E 0.3:DC-3F) | Significance† (*P* value) |
|---|---|---|---|---|---|---|
| Ellipticine | — | H | H | $1.6 \times 10^{-7}$ | 4 | < 0.05 |
| 9-OH-ellipticine | — | H | OH | $3 \times 10^{-7}$ | 10 | < 0.01 |
| 9-$CH_3O$-ellipticine | — | H | $CH_3O$ | $3 \times 10^{-7}$ | 4 | < 0.01 |
| 9-$NH_2$-ellipticine | — | H | $NH_2$ | $3.7 \times 10^{-7}$ | 15 | < 0.05 |
| 9-Br-ellipticine | — | H | Br | $1.6 \times 10^{-6}$ | 2.3 | < 0.01 |
| 2-$CH_3$-ellipticinium | $CH_3$ | H | H | $2.4 \times 10^{-6}$ | 10 | < 0.05 |
| 2-$CH_3$-9-OH-ellipticinium (NMHE) | $CH_3$ | H | OH | $1.2 \times 10^{-6}$ | 16 | < 0.05 |
| 2-$CH_3$-9-$NH_2$-ellipticinium | $CH_3$ | H | $NH_2$ | $4.9 \times 10^{-6}$ | 10 | < 0.01 |
| 2-$CH_3$-9-Br-ellipticinium | $CH_3$ | H | Br | $1.3 \times 10^{-6}$ | 5.3 | < 0.05 |
| 2,6-$(CH_3)_2$-9-OH-ellipticinium | $CH_3$ | $CH_3$ | OH | $6.4 \times 10^{-7}$ | 16 | < 0.01 |
| 2-$CH_3$-9-$CH_3$-COO-ellipticinium | $CH_3$ | H | $CH_3COO$ | $1.7 \times 10^{-6}$ | 21 | < 0.01 |
| Compound A‡ | | | | $2.5 \times 10^{-6}$ | 5.5 | ND |
| Compound B‡ | | | | $1.7 \times 10^{-7}$ | 19 | < 0.05 |

*Each ED50 value is an average of values from 2–6 independent experiments.

†Significance was calculated as described in the Materials and Methods section. ND = not determined because only one experiment was available.

‡Described in the Materials and Methods section.

Dactinomycin (Lyovac Cosmegen) was obtained from Merck, Sharp, and Dohme, Inc; daunorubicin and amethopterin from Specia; mitomycin from Choay; mithramycin from Charles Pfizer and Co; vincristine (Oncovin) from Eli Lilly and Co; and ethidium bromide from Sigma. Ethidium dimer has been described by Gaugain et al (20); it was provided by Dr. B. P. Roques.

### Assay of Drug Sensitivity

The sensitivity of the parental and resistant cell lines to the different drugs was determined as described by Biedler and Riehm (18), with the following modifications: 16-mm wells of a 24-well Costar dish (Costar, Cambridge, MA; No. 3524), containing 1 ml of MEM with graded drug concentrations, were inoculated with 1 ml of cell suspension containing either $2 \times 10^4$ parental cells or $4 \times 10^4$ cells of one of the resistant sublines. In these conditions the cells were growing exponentially, and the numbers of cells in the control cultures at the end of the experiment were about the same. Duplicate assays were carried out at each drug concentration. For each drug and each cell line, assays were performed at least two times. After 72 ($\pm$ 2) hours of drug exposure, the medium was removed, and the cultures were trypsinized and pipetted sufficiently to produce a single-cell suspension. After appropriate dilution in growth medium, the number of cells per well was determined by means of a Cytograf, model 6300A (Bio/Physics Systems, Inc).

The average number of cells in each treated culture was expressed as a percent of the average number of cells in the control cultures without drug. The ED50 value, defined as the dose effective in inhibiting and/or killing 50% of the cell population after 3 days of exposure to the drug, was obtained by plotting the percent of residual cells versus the drug concentration on a semilogarithmic scale. The ED50 value was determined from a linear regression with the aid of a computer. Significance of difference of response to drugs between sensitive and resistant cells was based on the $t$ test for independent random samples.

### Tumorigenicity

Tumorigenicity of the cell lines was determined by inoculation on the chorioallantoic membrane of embryonated chick eggs, as described by Barski et al (21,22). White Leghorn embryonated chick eggs at Day 12 of incubation were used. For each cell line, the assay was carried out at four different cell concentrations, ranging from 1 to $4 \times 10^6$ cells, injected in a volume of 0.1 ml. For each cell concentration, 18 eggs were inoculated. After 7 days of incubation, the embryos were killed, and the presence of malignant growth was determined by histologic examination of the eventual nodules.

Our cell lines were also tested for their ability to form colonies on agar plates. The plates consisted of 1% Noble agar (Difco) in $\alpha$ complete medium (23) containing 10% fetal calf serum. The agar plate culture technique has been described by Kuroki (24).

### Karyotype Studies

Chromosome preparations were made by generally standard procedures. After 2 hours of incubation with colchicine (1 $\mu$g/ml), the cells were harvested by trypsinizing the entire culture. G-banding preparations were made by treating the slides with 0.1% (wt/vol) trypsin in $Ca^{2+}$- and $Mg^{2+}$-free phosphate buffered saline and staining with Giemsa (25). Determination of the number of chromosomes per cell was made by examination of 50 metaphases for each cell line. For karyotype analysis, chromosomes were arranged as described by Worton et al (26).

### Determination of NMHE Cellular Uptake

Uptake of exogenous NMHE was followed by exposing cultures to the drug carrying a $^{14}C$-labeled methyl group at position 2 (2 millicuries/mM), synthesized and provided by Dr. Van Bac (27). Prior to the experiments, the resistant sublines were grown without drug for at least 10 days. The cells were plated 24 hours before the assay on a 3.6-$cm^2$ glass lamella in a Leighton tube containing 2 ml of growth medium. The number of plated cells was adjusted to about $1 \times 10^6$/tube at the time of drug exposure. After drug treatment in the conditions given for each experiment, the cells were washed three times with 4 ml of methanol at 4°C. There was no detectable radioactivity above background level in the final wash, thus demonstrating the effectiveness of this washing procedure, which gave the best results when compared to other washing solutions such as medium, phosphate-buffered saline, or 0.9% NaCl. The monolayer was then solubilized by incubation for at least 2 hours at 37°C in 1 ml of 0.1 M NaCl, 0.01 M Tris-HCl (pH 7.4), 0.001 M EDTA, and 0.5% sodium dodecylsulfate, as described by Johnson et al (28). The cell lysate was then transferred to a scintillation vial containing 7 ml of toluene-Triton-based scintillation fluid, and the radioactivity was determined in an Intertechnique SL 32 counter. The tube was rinsed with 1 ml of methanol, which was counted separately. Usually the amount of residual

radioactivity in the rinsing did not exceed 5% of the sample radioactivity. Each determination was carried out in duplicate.

### Uptake of Dactinomycin

The cells were plated 24 hours before the assay in 35-mm Petri dishes containing 2 ml of MEM. The number of cells was about $1 \times 10^6$/dish at the time of drug exposure. The cells were then exposed to ($^3$H)-dactinomycin (2 microcuries/ml; 2 μg/ml), and the uptake of the drug was determined as previously described (29).

## RESULTS

### Selection of Resistant Sublines

Figure 1 shows the effects of different 9-OH-E concentrations on the growth of the parental sensitive line DC-3F. The cellular growth was significantly decreased when the drug was present in the medium at a concentration of 0.04 μg/ml and completely inhibited at 0.12 μg/ml. From this strain, drug-resistant sublines were selected by adding stepwise increasing concentrations of drug to the cell growth medium. The cells were grown successively in the presence of 0.05, 0.10, 0.15, and 0.3 μg/ml of 9-OH-E. At each concentration, the cells were maintained until all the culture was growing well. Then the next step was initiated with an inoculum of about $1 \times 10^6$ cells. After about 20 months of drug exposure in these conditions, a subline was established which was able to grow normally in the presence of 0.3 μg/ml of 9-OH-E. This subline, designated DC-3F/9-OH-E 0.3, was the first one used in the experiments described in this paper. From this subline, five independent clones were isolated which displayed identical properties. DC-3F/9-OH-E 0.3 was then successively grown in the presence of 0.4 and 0.6 μg/ml of 9-OH-E. After about 4–5 months, a subline resistant to this drug concentration was obtained and designated DC-3F/9-OH-E 0.6.

The 9-OH-E ED50 values of parental and resistant cells were determined by the 3-day assay procedure (fig 2), and the level of resistance of the different sublines was defined by the ratio R (ED50 in resistant subline/ED50 in DC-3F). From this estimation, the DC-3F/9-OH-E 0.3 and DC-3F/9-OH-E 0.6 sublines are respectively tenfold and 12-fold resistant to 9-OH-E. It is interesting to note that after > 30 months of drug exposure (up to 1 μg/ml), we have not been able to establish any subline with a higher level of resistance.

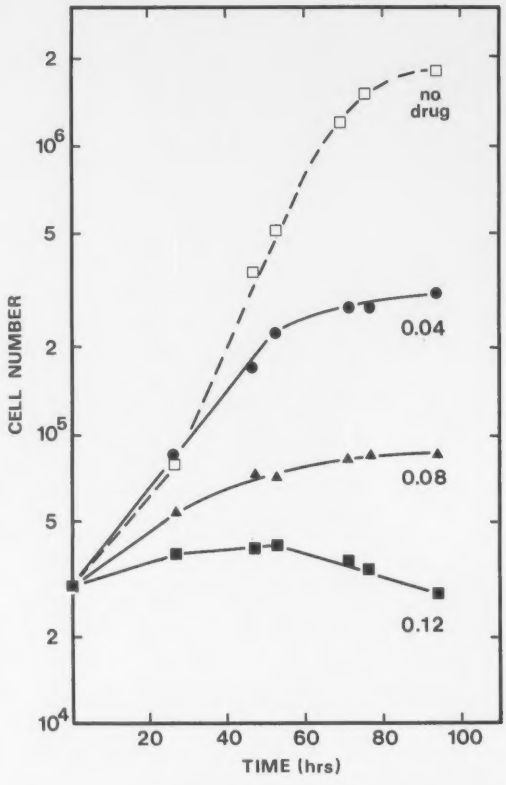



FIGURE 1.—DC-3F growth inhibition by 9-OH-E at different concentrations. $3 \times 10^4$ cells were plated in 16-mm wells of a 24-well Costar Dish containing 2 ml of medium at the indicated drug concentrations (μg/ml). At the indicated times, the medium was removed and the cells were then counted with a Cytograf model 6300A. Cell counts were carried out in duplicate.

### Stability of the Resistance

The DC-3F/9-OH-E 0.3 subline was grown for 18 months in the absence of drug in the culture growth medium. Once every 2 months, the 9-OH-E ED50 value was determined for these cells and compared with the value obtained for the cells which were maintained in the presence of the drug. For this period of time, the ED50 values remained constant and identical in both cases, thus indicating that, in this subline, the resistance to 9-OH-E is stable even in the absence of selection.

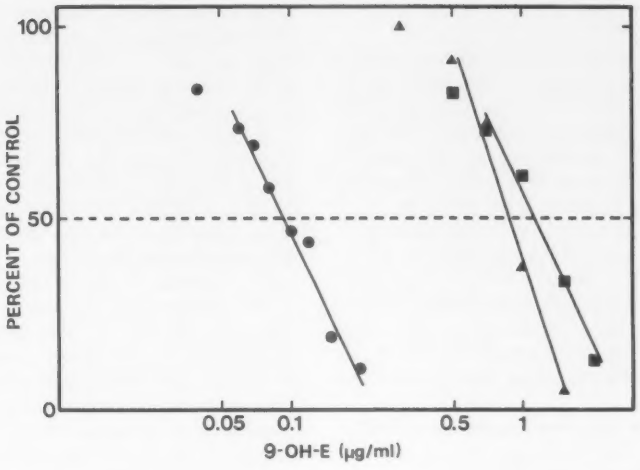



FIGURE 2.—Response of parental DC-3F and 9-OH-E-resistant sublines to various concentrations of 9-OH-E. The cells were exposed to the different drug concentrations in the conditions described in the Materials and Methods section. Each point is an average of a duplicate determination. ● = DC-3F; ▲ = DC-3F/9-OH-E 0.3; ■ = DC-3F/9-OH-E 0.6.

## Growth and Morphologic Characteristics of the Resistant Sublines

Established resistant sublines were grown routinely in the presence of the corresponding drug concentration and transferred every 4–5 days. During the development of resistance to 9-OH-E, marked differences in cell morphology and patterns of growth in culture became apparent. Table 2 shows some properties of the parent and resistant sublines. Increasing resistance levels were associated with increased doubling times, which were identical whether measured in the presence or in the absence of the drug. The resistant cells tend to grow more slowly than the parental cells, and even in the absence of drug, display a decreased plating efficiency.

The resistant cells undergo striking morphologic modifications. Both DC-3F/9-OH-E 0.3 and DC-3F/9-OH-E 0.6 sublines are more flattened and grow in compact arrays to finally form dense layers of contiguous cells. The parent DC-3F line grows as a disordered network and, at confluence, forms multilayer aggregates of cells with weak adhesion to the substrate. The saturation density reached by the DC-3F line is almost twice that of the resistant sublines.

TABLE 2.—Properties of the parental DC-3F and 9-OH-E-resistant sublines

| Cell line | 9-OH-E maintenance concentration (μg/ml) | ED50* (μg/ml) | Population doubling time (hrs) | Plating efficiencies (%) | | |
|---|---|---|---|---|---|---|
| | | | | No drug | 0.3 μg/ml† | 0.6 μg/ml† |
| DC-3F | — | 0.1 | 13.0 | 78 ± 7 | 0 ± 0 | 0 ± 0 |
| DC-3F/9-OH-E 0.3 | 0.3 | 1 | 18.0 | 66 ± 6 | 49 ± 5 | 3 ± 2 |
| DC-3F/9-OH-E 0.3‡ | — | 1 | 18.0 | 74 ± 7 | 47 ± 5 | — |
| DC-3F/9-OH-E 0.6 | 0.6 | 1.2 | 24.0 | 57 ± 7 | 54 ± 5 | 38 ± 7 |

*Average of 3 independent determinations.
†9-OH-E concentration in the cell growth medium.
‡Maintained without drug for 18 mos.

## Tumorigenicity

Drug-sensitive and drug-resistant cells were tested for their ability to form tumors after injection on the chorioallantoic membrane of the chick embryo. Figure 3 shows that, after injection of DC-3F/9-OH-E 0.3 cells at different concentrations, the percent of tumor induction was about 80% lower than after injection of the same number of DC-3F cells.

A number of studies have shown that cloning efficiency in semisolid agar is the in vitro property which correlates best with in vivo tumorigenicity (30–32). We therefore examined the ability of both types of cells to grow in soft agar: the sensitive line formed colonies with an efficiency of about 60%, as compared to about 10% on plates seeded with the resistant cells.

These results show that the resistance to 9-OH-E in these cell lines is associated with a marked decrease of the oncogenic potential.

## Cytogenetic Studies

Determination of the number of chromosomes per cell was made on each subline after the establishment of the level of resistance. The modal number for the parental DC-3F line and both resistant sublines was 20 ± 1, the diploid chromosome number of the Chinese hamster being 22. In all cases, the frequency of polyploid cells was very low.

Figure 4 shows representative karyotypes of the parental line and the two sublines resistant to 0.3 and 0.6 μg/ml of 9-OH-E. The karyotypes of the resistant strains are identical to one another, but they differ from the karyotype of the sensitive line. Several abnormalities can be observed among the smaller chromosomes, Nos. 6–11: in particular, > 70% of the metaphases examined exhibited small arm deletions of No. 8 chromosomes, and numerical changes were frequently observed in Nos. 9–11. However, the most striking and consistent modifications were observed among the larger chromosomes. In most cases, chromosomes 1 and 2 were exempt from alterations. One of the X chromosomes was usually absent from both parental and resistant cells, and one of the No. 5 chromosomes was absent from resistant cells while it was present in about 60% of the sensitive cells. Finally, an additional chromosome, which was called M chromosome, was present in all metaphases of resistant cells. This large metacentric chromosome displays a characteristic G-banding pattern, which is different from any other chromosome in the cell.

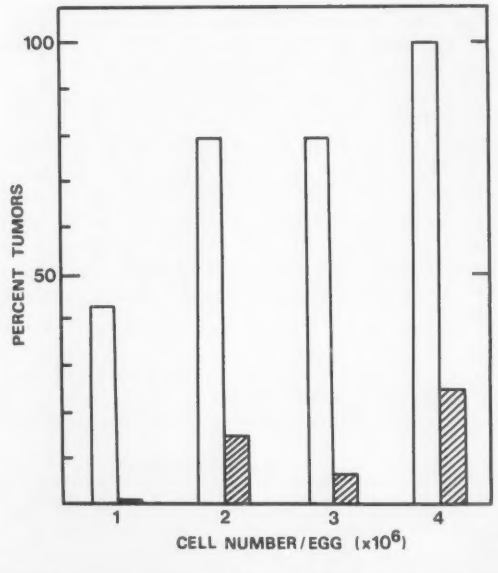



FIGURE 3.—Tumorigenicity of the parental DC-3F and DC-3F/9-OH-E 0.3 subline. Each cell line was tested for tumor induction after injection on the chorioallantoic membrane of the chick embryo, as described in the Materials and Methods section. Open bars = DC-3F; hatched bars = DC-3F/9-OH-E 0.3.

## Cross-resistance With Other Antitumor Agents

The response of parental and resistant cells to different antitumor agents was determined by the 3-day assay procedure. Table 1 shows the results obtained with some ellipticine derivatives. The 9-substituted molecules all have about the same cytotoxicity on the DC-3F line, with the exception of 9-Br-ellipticine (9-Br-E), which is about ten times less cytotoxic than ellipticine. The DC-3F/9-OH-E 0.3 subline is cross-resistant to these molecules to various extents: cross-resistance to 9-$NH_2$-ellipticine is approximately the same as the resistance to the selective agent itself, whereas it is only fourfold for 9-$CH_3O$-ellipticine and ellipticine and about twofold for 9-Br-E. It is interesting to note that, in contrast to all other molecules in this series, 9-Br-E does not intercalate in DNA.

Quaternary ammonium compounds, carrying a substitution at position 2, were then examined. When compared to their uncharged counterparts, these derivatives displayed an identical or lower cytotoxicity on the parental sensitive line, while the resistance levels on the DC-3F/9-OH-E 0.3 subline were generally higher. Yet, the resistance to 2-$CH_3$-9-Br-ellipticinium remains markedly lower than that to the other ellipticinium derivatives.

FIGURE 4.—Representative karyotypes of parental and 9-OH-E-resistant sublines. Chromosomes are arranged as described by Worton et al (20).

TABLE 3.—Cytotoxicity of some antitumor agents against the parental DC-3F line and the DC-3F/9-OH-E 0.3 subline

| Drugs | MW | ED50 for DC-3F (M)* | ED50 ratio (DC-3F/9-OH-E 0.3:DC-3F) | Significance† (*P* value) |
|---|---|---|---|---|
| Ethidium bromide | 427 | $8.8 \times 10^{-7}$ | 11 | < 0.05 |
| Daunorubicin | 527 | $1.3 \times 10^{-8}$ | 8.5 | < 0.05 |
| Vincristine (sulfate) | 923 | $3.2 \times 10^{-8}$ | 7.3 | < 0.05 |
| Dactinomycin | 1255 | $7.0 \times 10^{-10}$ | 7 | < 0.05 |
| Ethidium dimer | 856 | $5.8 \times 10^{-7}$ | 4.4 | < 0.05 |
| Amethopterin | 454 | $3.6 \times 10^{-8}$ | 3 | < 0.05 |
| Mithramycin | 1089 | $4.3 \times 10^{-8}$ | 2.2 | < 0.05 |
| Mitomycin | 334 | $1.0 \times 10^{-7}$ | 1.6 | NS |

*Each value is an average from 2–6 independent experiments.
†Significance was calculated as described in the Materials and Methods section; NS = not significant.

Table 1 also shows that the 7*H*-pyridocarbazole derivative (compound A; defined in Materials and Methods), which is a structural analog of ellipticine, exhibits about the same cytotoxicity on the DC-3F line. The dimer derivative (compound B), which is endowed with a DNA affinity about 100-fold higher than that of the monomer, is about 30 times more cytotoxic than the monomer.

These results diverge in several respects from those previously reported by Paoletti et al (16), who studied the activity of the ellipticine derivatives on L1210 mouse leukemia in vitro. In that system, a 1000-fold difference was observed between the cytotoxic effects of the most (9-OH-E) and the least (9-Br-E) active compounds, and a correlation was observed between the cytotoxicity and the DNA affinity of the drugs in the series. In our experimental system, there was only about a 15-fold difference between the most and least cytotoxic molecules, and there was no relationship between cytotoxicity and DNA affinity. Whether these discrepancies are related to such problems as drug metabolism and/or cellular uptake remains to be studied.

The same cytotoxicity assay was also used to determine the sensitivity of these cell lines to other antitumor agents with various structures and mechanisms of action. Table 3 shows that, in our system, ellipticine derivatives are much less cytotoxic than most other antitumor drugs. The DC-3F/9-OH-E 0.3 subline displays some cross-resistance to all these agents except mitomycin. A low, but significant, cross-resistance was observed with mithramycin, amethopterin, and the ethidium dimer, a bis-intercalating agent consisting of 2 ethidiums linked by an aminoalkyl chain (20). The cross-resistance to dactinomycin, vincristine, daunorubicin, and ethidium bromide was approximately the same as the resistance to 9-OH-E. Cross-resistance between DNA intercalating agents and vinca alkaloids has previously been reported (18,33). In our cell lines, there was no correlation between the level of cross-resistance and the molecular weight (MW) of the drugs. For example, the resistance to ethidium dimer (MW = 856) is much lower than to the monomer (MW = 427). This is in contrast to the results reported by Biedler and Riehm (18) on a DC-3F subline resistant to high levels of dactinomycin. Although not cross-resistant to several drugs of similar molecular weights (18), this dactinomycin-resistant subline (DC-3F/AD X) was, in our hands, about tenfold resistant to 9-OH-E (MW = 298).

## Uptake of Ellipticine Derivatives

Drug uptake by the parental line and the resistant sublines was measured by exposing the cells to a radiolabeled 9-OH-E derivative, NMHE. 9-OH-E-resistant cells were cross-resistant to this drug to about the same extent as to 9-OH-E (table 1). Figure 5 illustrates the time course uptake of ($^{14}$C)-NMHE in sensitive and resistant cell lines. After a rapid increase during the first 30 minutes, the drug uptake tends to reach a plateau after about 2 hours. This figure shows that there is no significant difference in the kinetics of NMHE uptake by the sensitive and resistant cells.

Finally, the cell lines were exposed for 3 hours to the drug at different concentrations. In these conditions, the uptake of NMHE was proportional to the drug concentration in the cell growth medium, up to 8 μg/ml. Again, in these experiments, there was no significant difference between the sensitive and resistant cells (data not shown).

Identical experiments were also carried out with 2-*N*-$CH_3$-ellipticinium, a fluorescent derivative of ellipticine. After extraction from the cells with ethyl acetate in the presence of tetraphenylboron, 2-*N*-

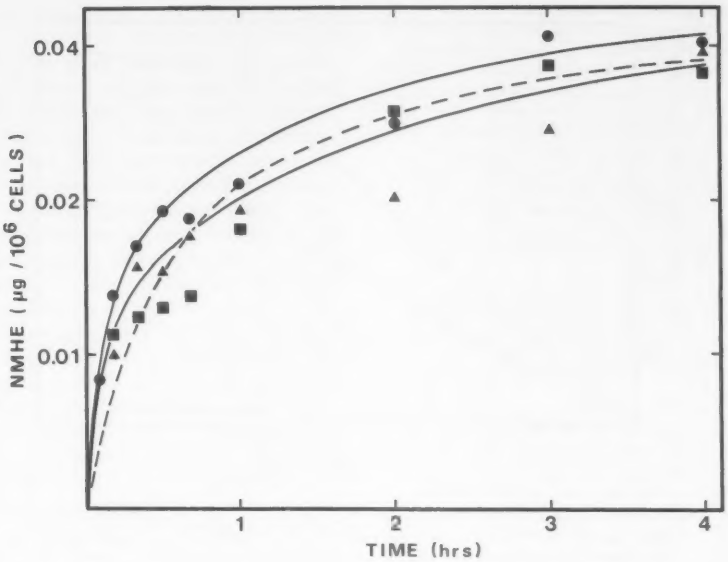



FIGURE 5.—Time-course of the uptake of ($^{14}$C)-NMHE by the parental and 9-OH-E-resistant cells. 1 × $10^6$ cells were incubated with the drug (1 μg/ml; 2 microcuries/μM) and at the indicated times, the drug concentration was determined as described in the Materials and Methods section. ●——● = DC-3F; ▲——▲ = DC-3F/9-OH-E 0.3; ■——■ = DC-3F/9-OH-E 0.6.

$CH_3$-ellipticinium concentration was determined by high-performance liquid chromatography coupled to a fluorescence detector. Results similar to those reported above with the radioactive derivative were obtained.

### Uptake of Dactinomycin

As shown in table 3, 9-OH-E-resistant cells are cross-resistant to dactinomycin to about the same extent as to 9-OH-E. On the other hand, DC-3F/AD X was found to be about tenfold resistant to 9-OH-E. Figure 6 shows the results of ($^3$H)-dactinomycin uptake studies by DC-3F and the 9-OH-E or dactinomycin-resistant cells. After 3 hours of incubation in the presence of 2 μg/ml of antibiotic, the uptakes by DC-3F/9-OH-E 0.6 and DC-3F/AD X were, respectively, 55% and 4% of the uptake by the sensitive line. The dactinomycin uptake by DC-3F/9-OH-E 0.3 was identical to the uptake by DC-3F/9-OH-E 0.6. In contrast, NMHE uptake by DC-3F/AD X was not modified. This indicates that the resistance of the DC-3F/9-OH-E 0.3 and DC-3F/9-OH-E 0.6 cells to dactinomycin is associated with either a decreased drug retention or an impaired permeability. However, it was shown by Biedler and Riehm (18) that the resistance of the DC-3F/AD X subline results from a decreased permeability to the drug.

## DISCUSSION

In this paper, we describe the isolation and some properties of Chinese hamster lung cells resistant to 9-OH-E. These sublines were selected by growing DC-3F cells under stepwise increasing concentrations of drug in the cell growth medium. Development of drug resistance appeared as a slow process, and despite > 30 months of drug exposure, the level of resistance, estimated from the ratio of the ED50 in the resistant cells to the ED50 in the sensitive cells, never exceeded a value of 10–12. This was somewhat unexpected since sublines resistant to dactinomycin or daunorubicin, two other DNA-intercalating antitumor agents isolated from the same parental line, were endowed with several hundredfold resistance levels.

Cytogenetic analysis revealed that the karyotype of 9-OH-E-resistant cells carried several discrete modifications, the most striking being the presence

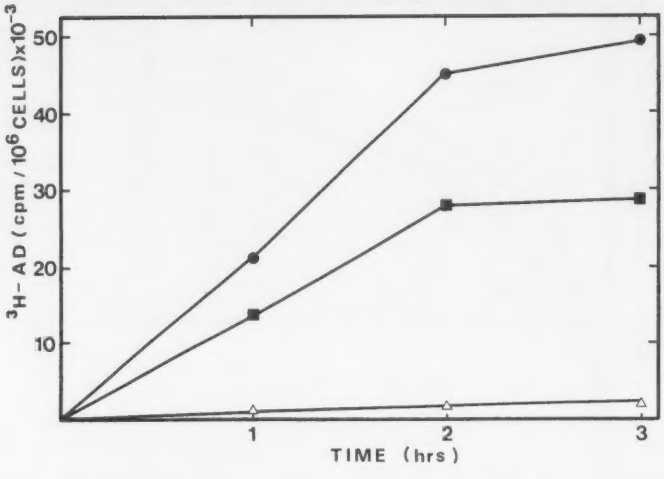



FIGURE 6.—Uptake of ($^3$H)-dactinomycin (AD) by DC-3F (●), DC-3F/9-OH-E 0.6 (■), and DC-3F/AD X (△) cells. The experimental conditions are described in the Materials and Methods section.

of a marker chromosome which is characterized by a peculiar G-banding pattern. However, this pattern does not make it possible to define precisely the origin of this chromosome, which might arise from a complex rearrangement of several fragments from other chromosomes. The 9-OH-E-resistant cells, which were grown for about 18 months in the presence of 9-OH-E, display a markedly constant karyotype. Whether the M chromosome, as well as the other observed chromosome changes in the resistant cells, are induced by the drug treatment or correspond to the karyotype of a very small fraction of the DC-3F population, which is selected when the cells are grown in the presence of the drug, is still unknown. Biochemical and genetic studies are now in progress in our laboratory to analyze the possible role of this chromosome in the mechanism of resistance.

The 9-OH-E-resistant cells showed a reduced capacity to form tumors after injection on the chorioallantoic membrane of the chick embryo and a markedly decreased ability to grow in vitro on semisolid medium. In addition, the cells exhibited morphologic and growth parameter modifications, such as a different cell doubling time, which increase with the cell resistance level. These results show that, when acquiring resistance to 9-OH-E, the spontaneously transformed DC-3F line tends to regain a less malignant phenotype. Identical results have previously been reported with the DC-3F variants that are highly resistant to dactinomycin (34). The precise relationship that accounts for the simultaneous development of resistance to these antitumor agents and the reduction of oncogenic potential remains to be studied. On the other hand, as previously discussed by Brockman (35), this association of resistance and reduction of malignancy might be of clinical significance.

As an approach to the understanding of the mechanism of resistance, the uptake of different drugs by DC-3F and dactinomycin- or 9-OH-E-resistant sublines was measured. The results show that uptake of ellipticine derivatives by 9-OH-E- or dactinomycin-resistant cells is not modified, while, as already reported in a similar system by Biedler et al (34), the resistance to dactinomycin is associated with a reduced cell permeability to the drug, the extent of which depends on the level of resistance. This suggests that the mechanism of resistance to 9-OH-E, and its derivatives, might not be related to an impaired permeability of the resistant cells to the drug. However, it should be noted that the technique used in these experiments allows measurement of the total binding of the drug to the cell but not determination of the true intracellular drug concentration. As discussed by Kohn et al (36), ellipticine derivatives are lipophylic molecules which are able to bind to the cell membranes. Therefore, to

lower the unspecific binding of the drug to the cells as much as possible, several solvents were tested to wash the cells after drug exposure. The results presented here are based on experiments carried out with methanol, which was the most drastic solvent. However, whatever the solvent was, the results were comparable, and there was no appreciable difference in the amounts of drug retained by the parental and the resistant cells. It is then possible that, from the amount of drug bound to the cell, only a fraction actually enters the cell, the rest remaining associated with the membrane. We might then observe an apparently equivalent uptake of the drug by the sensitive and resistant cells, whereas resistance would result from a significant difference in the amounts of drug able to reach the ultimate target, which is likely to be the intranuclear DNA. To elucidate this problem, a more detailed analysis of the mechanism of penetration of the ellipticine derivatives inside the cells is now in progress.

In conclusion, the results reported here show that the development of resistance to ellipticine derivatives is associated with some changes in cell properties, such as morphologic and growth parameter modifications, lower oncogenic potential, cross-resistance to a variety of antitumor agents, and decreased permeability to dactinomycin.

These modifications might be ascribed to an alteration of the cell membrane. Whether such a membrane alteration also results in an impaired permeability to 9-OH-E, which would account for the resistance of the cells to this drug, remains to be shown.

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# Biochemical Activation of AZQ [3,6-Diaziridinyl-2,5-bis(carboethoxyamino)-1,4-benzoquinone] to Its Free Radical Species[1]

Peter L. Gutierrez,* Rosalind D. Friedman, and Nicholas R. Bachur[2,3]

## SUMMARY

**The aziridinyl quinone 3,6-diaziridinyl-2,5-bis(carboethoxyamino)-1,4-benzoquinone is an antitumor agent that enhances electron flow from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to molecular oxygen in rat liver microsomes, rat liver nuclei, and purified NADPH-cytochrome c reductase. The process is enzymatic and has an optimum pH of 7.5; NADPH is the preferred cofactor. Electron spin resonance studies show the presence of a free radical semiquinone characterized by five hyperfine lines at g = 2.0046.**

[Cancer Treat Rep 66:339–342, 1982]

The aziridinyl quinone 3,6-diaziridinyl-2,5-bis(carboethoxyamino)-1,4-benzoquinone (AZQ) is a quinone-containing alkylating agent (fig 1) originally designed to cross the blood-brain barrier and to have increased antitumor activity on malignancies of the central nervous system (1). The drug has activity against intraperitoneal L1210 leukemia, P388 leukemia, and B16 melanoma (1), as well as intracerebral L1210 leukemia (2,3). At present, AZQ is undergoing phase I clinical trials, so it becomes increasingly important to understand its metabolism and cytotoxicity. In earlier studies, Schwartz et al (4) showed that microsomal reduction of the quinone-containing agent mitomycin to a hydroquinone was necessary for cytotoxic activity. Later, we observed that quinone drugs are reduced to a drug free radical intermediate, which we suggested was necessary for the cytotoxic activity (5). The drug free radical can also react with oxygen to produce superoxide, which may be involved in the cytotoxic effects of these agents. Because AZQ is a quinone-containing agent and because the aziridine ring is required but not sufficient for antitumor activity (1), we have investigated its enzymatic conversion to a free radical.

## MATERIALS AND METHODS

AZQ was supplied by the Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD), and was used without further purification. For oxygen measurement experiments, 1 mM stock solutions of the agent were prepared by dissolving it in dimethylsulfoxide at a final concentration of 0.6% and then by adding 0.2 M potassium phosphate buffer to achieve the required drug concentration. For electron spin resonance (ESR) experiments, stock solutions were prepared similarly in 0.6%–1% dimethylsulfoxide.

Microsomes from rat liver were prepared according to the method of Omura and Takesue (6). Prior to use in the oxygen electrode, the microsomes (0.7 mg of protein as measured by the Lowry method) were preincubated on ice for 15 minutes with 2% (vol/vol) Triton N-101 (Sigma Chemical Co, St. Louis, MO). This procedure lowers the endogenous oxygen consumption of microsomes (5).

Nuclei from rat liver were prepared according to

[1]Received Jan 29, 1981; revised July 31, 1981; accepted Aug 24, 1981.

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[3]We thank Ms. Lillian De Vaughn for her assistance with the oxygen consumption measurements.

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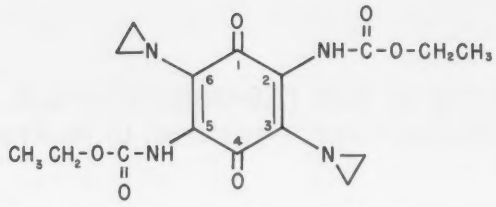


AZQ

FIGURE 1.—Chemical structure of AZQ.

the method of Kasper (7). In a typical reaction mixture, 2.3 mg of nuclear protein was used.

Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome c reductase was prepared by the method of Omura and Takesue (6).

### Oxygen Measurements

Oxygen uptake was determined with a Clark-type electrode in a Biological Oxygen Monitor (model 53, Yellow Springs Instrument Co). All measurements were made at 37°C. The reaction mixtures (final volume, 1 ml) contained 0.2 M potassium phosphate buffer (pH 7.5); 5 mM NADPH; an enzyme source (rat liver microsomes, nuclei, or purified enzyme); and $10^{-7}$ to $10^{-4}$ M AZQ. The buffer was aerated in the chamber for 3 minutes; microsomes, nuclei, or purified enzyme were added; and the electrode was placed in contact with the solution. After allowing 1 minute for equilibration, NADPH was injected into the reaction mixture. Endogenous oxygen uptake was monitored for 1 minute, AZQ was then introduced into the system, and AZQ-induced oxygen uptake was recorded for 3–5 minutes. Oxygen consumption evaluation was based on a 100% value of $1.58 \times 10^{-7}$ mols of dissolved oxygen.

### ESR Spectroscopy

ESR measurements were made with an X-band (9.3 GHz) Varian E-109 Century series spectrometer equipped with 100 kHz field modulation. A dual cavity (TE 104) was used, which contained a strong pitch marker ($g = 2.0028$) in one section and the sample in an aqueous ESR cell in the other. The strong pitch marker was used to evaluate g values. Reaction mixtures contained 0.2 M potassium phosphate buffer, pH 7.5; 5 mM NADPH; an enzyme source (rat liver microsomes or NADPH-cytochrome c reductase); and 1 mM AZQ. Aerobic samples (1 ml) were run without additional air bubbling or exposure. The 1-ml anaerobic samples were prepared in sealed test tubes by purging with nitrogen for 2–3 minutes. The reaction mixture was transferred to a previously nitrogen-purged aqueous ESR cell immediately after injection of the enzyme source. All measurements were performed at room temperature with incident microwave power ≤ 10 mW and a modulation amplitude of 1.0–0.01 gauss.

## RESULTS AND DISCUSSION

When incubated with NADPH-cytochrome c reductase, rat liver microsomes, or rat liver nuclei, AZQ enhances NADPH-dependent oxygen consumption in a reaction that is saturable by the drug and is linear in response to protein concentration (fig 2). At lower protein concentrations, there seems to be a slight deviation from linearity; yet in the absence of enzyme, no oxygen is consumed. The pH profile in potassium phosphate buffer (fig 3) shows a maximum at pH 7.5. Experiments with Tris buffer gave lower reaction rates, similar to our experience with quinone-containing antitumor agents (5). NADPH is the preferred cofactor for the three enzyme sources (table 1). The data fit enzyme kinetic equations that allow for the determination of the kinetic constants $K_m$ and $V_{max}$ with the drug as a substrate (table 2). The $V_{max}$ of the purified enzyme is several orders of magnitude higher than that obtained from microsomes and nuclei. $K_m$ values, on the other hand, are all of the same order of magnitude.

A free radical intermediate was detected by ESR when anaerobic or aerobic solutions of NADPH, AZQ, and potassium phosphate buffer (pH 7.5) were reacted with rat liver microsomes or NADPH-cytochrome c reductase (fig 4). No free radical was detected in the absence of enzyme or AZQ. Both anaerobic and aerobic conditions show a free radical within 2 minutes of mixing, which is the time required for transferring the mixture to the spectrometer. With anaerobiosis at 25°C, the free radical decays in 3–5 minutes.

The five-line ESR spectrum of relative intensities 1:2:3:2:1 has a g value of 2.0046, with line widths of

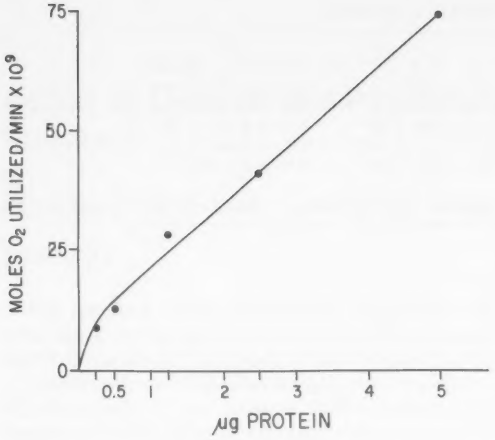


FIGURE 2.—Linearity of $O_2$ consumption as a function of enzyme concentration. Conditions at 37°C are: $5 \times 10^{-5}$ M AZQ; 5 mM NADPH; 0.2 M potassium phosphate buffer, pH 7.5; and NADPH-cytochrome c reductase.

approximately 1 gauss (fig 4). Additional hyperfine structure could not be obtained, even at modulation amplitudes $\leq 0.5$ gauss. The spectrum is, in all probability, that of the AZQ semiquinone free radical, with the unpaired electron delocalized on the π-system of the quinone ring.

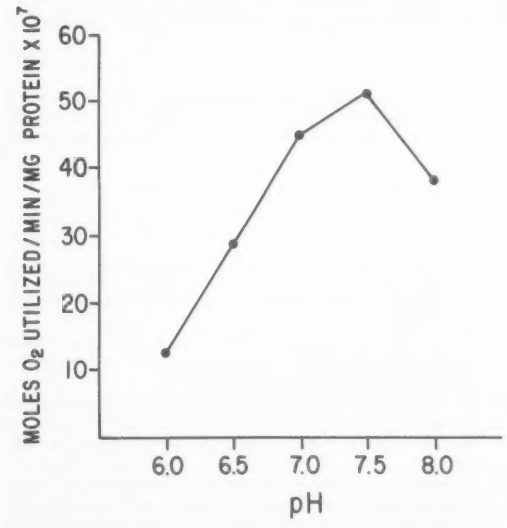


FIGURE 3.—pH profile of the $O_2$ consumption rates with NADPH-cytochrome c reductase and $5 \times 10^{-5}$ M AZQ. Other conditions are as described in the Materials and Methods section.

TABLE 1.—Cofactor preference

| Enzyme source and cofactor | $O_2$ utilized (mols/min/mg of protein) |
|---|---|
| Microsomes | |
| NADPH | $13 \times 10^{-8}$ |
| NADH* | $9.4 \times 10^{-8}$ |
| NADPH-cytochrome c reductase | |
| NADPH | $5.1 \times 10^{-6}$ |
| NADH | 0 |
| Nuclei | |
| NADPH | $1.7 \times 10^{-8}$ |
| NADH | $0.3 \times 10^{-8}$ |

*NADH = reduced nicotinamide adenine dinucleotide.

With this arrangement, one would expect an ESR spectrum that shows coupling of the unpaired electron to four nitrogens and two β-protons (two bond lengths away from the unpaired electron) on the alkylamino groups. The least complicated spectrum with this arrangement would be one in which the nitrogens as well as the β-protons are magnetically equivalent and have identical couplings. This would yield 11 lines that we do not see. The five lines we detect with the relative line intensities given above can be attributed to the interaction of the unpaired electron with two magnetically equivalent nitrogen nuclei. The coupling of this interaction is approximately $A_N = 1.8$ gauss. It is difficult at this point to state which two of the four nitrogens are the ones producing the coupling. If the coupling were due to the nitrogens in the alkylamino group, there is no reason to believe that the β-protons would not also interact. On the other hand, all four nitrogens could show couplings, two of which could be considerably smaller than the other two. In this case, the smaller couplings may be difficult to detect. Equally difficult to detect would be the coupling to γ-protons (three bond lengths away). These couplings, however small, may contribute to the line width. It is also possible that we are not seeing all of the lines in the spectrum because of the spectrum's relative weakness.

In conclusion, we have shown that AZQ stimulates the consumption of oxygen by microsomes, nuclei, and purified NADPH-cytochrome c reductase. When compared with other quinone-containing agents such as daunorubicin, doxorubicin, and mitomycin (5,8), AZQ appears to be a preferred substrate for this enzyme because its $K_m$ is 100-fold smaller (eg, $10^{-4}$ vs $10^{-6}$ M).

We have shown that a free radical intermediate of AZQ is produced on biochemical reduction of the drug. The free radical is probably that of AZQ semiquinone, showing coupling of two magnetically equivalent nitrogens. We have yet to determine the nitrogens responsible for the coupling and whether

TABLE 2.—Kinetics of AZQ-induced $O_2$ consumption*

| Enzyme source | Optimum pH | Cofactor preference | $K_m$* (M) | $V_{max}$* (mols of $O_2$ utilized/ min/mg of protein) |
|---|---|---|---|---|
| Nuclei | 7.5 | NADPH | $1.7 \pm 0.34 \times 10^{-6}$ | $0.18 \pm 0.034 \times 10^{-7}$ |
| Microsomes | 7.5 | NADPH | $5.7 \pm 1.7 \times 10^{-6}$ | $1.7 \pm 0.82 \times 10^{-7}$ |
| NADPH-cytochrome c reductase | 7.5 | NADPH | $2.1 \pm 1.0 \times 10^{-6}$ | $100 \pm 5 \times 10^{-7}$ |

*Values = mean ± SE.

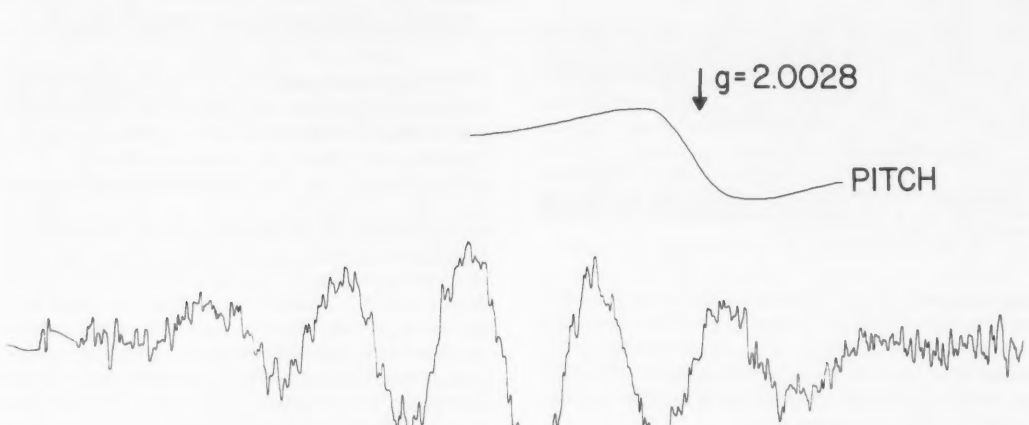


FIGURE 4.—ESR spectrum of AZQ free radical anion formed in anaerobic 1-ml incubations of 5 mM NADPH; 1 mM AZQ; 0.2 M potassium phosphate buffer (pH 7.5); and NADPH-cytochrome c reductase (3.5 mg). Identical spectra were obtained with incubation mixtures containing rat liver microsomes (0.7 mg). ESR conditions at room temperature were 9.3 GHz, 10-mW incident microwave power, and 0.5-gauss modulation amplitude.

this signal is from the parent drug, a metabolic intermediate, a metabolic product, or some combination.

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# Action of Lycurim and Pyrazofurin on Hepatoma 3924A Cells in Culture[1,2]

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## SUMMARY

**The purpose of this study was to test for a possible synergism in the antitumor action of the antimetabolite pyrazofurin (PF) and the alkylating agent lycurim (LY) [1,4-di-(2′-mesyloxy-ethylamino)-1,4-dideoxy-*m*-erythritol dimethylsulfonate] on proliferating and resting rat hepatoma 3924A cells in tissue culture. Earlier studies showed that PF killed hepatoma cells only in the logarithmic phase of growth as determined by colony-forming ability. Now we report that LY killed hepatoma cells in both the exponential and plateau phases. Treatment of cultures in each phase for 1 or 2 hrs resulted in threshold exponential type dose-response curves, yielding $D_o$ values of 3 and 1 μM, respectively. LY is a cycle-specific drug, although fluctuations in cell age-dependent survival were also demonstrated. In synchronized cultures, cells were most sensitive to 0.1 μM LY in early $G_1$ phase, but in higher concentration (5 μM), LY killed the cells during the entire cell cycle, especially in $G_1$, early and mid S phases, and M phase. Lycurim and PF given together in their IC50 concentrations (0.016 and 0.085 μM, respectively) yielded synergistic cell kill in 7-day treatment of proliferating cultures. Two-hour treatment of exponentially growing cells with LY and PF at their IC50 concentrations (1 and 20 μM, respectively) also yielded synergistic killing action, but in plateau-phase cells only summation was observed. Synergistic activity of LY and PF in hepatoma 3924A cells was dependent on drug concentration and incubation time.**

[Cancer Treat Rep 66:343–349, 1982]

Lycurim (LY), 1,4-di-(2′-mesyloxy-ethylamino)-1,4-dideoxy-*m*-erythritol dimethylsulfonate, the sugar derivative dimethylsulfonate, was synthesized in Hungary as part of a series of anticancer ethylamino-sugar alcohols (1–4). LY is an alkylating agent that inhibited the growth of several transplantable tumors of mice and rats over a wide dose range (1–4). In clinical trials, LY had antitumor activity on lymphoid tumors and on some solid neoplasms (2,3). It decreased the number of dividing cells in experimental tumors, causing a premitotic block. It inhibited DNA synthesis with little action on RNA or protein synthesis (4). A synergistic response was observed in L1210 leukemia cells in mice treated with LY in sequential combination with cyclophosphamide (5). Recent work in this laboratory showed that LY inhibited transaldolase activity in extracts of solid hepatoma 3924A.[6]

Pyrazofurin (PF) is an antimetabolite inhibitor of

[1]Received Feb 2, 1981; revised July 2, 1981; accepted July 20, 1981.

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[6]Weber G, and Prajda N. Unpublished observation.

de novo pyrimidine biosynthesis (6) when it is converted to its active form, 5′-monophosphate, by adenosine kinase, yielding a competitive inhibitor of orotidylate decarboxylase (6–10). PF caused a growth inhibition of rodent tumors (6,11) and was also tested clinically (8–10). It was suggested for use in combination chemotherapy (7,9,12,13). PF plus galactosamine caused a synergistic decrease of uridine-5′-triphosphate and cytidine-5′-triphosphate pools of hepatoma cells, both in vivo and in vitro (12,14,15). In tissue culture PF was phase-specific, killing hepatoma cells only in their logarithmic phase (13–15).

On the basis of these data, it was of interest to test whether PF and LY might be effective agents in combination chemotherapy. The present work was directed to elucidate the combined effect of PF and LY on the survival of proliferating and resting hepatoma 3924A cells. For this purpose the lethal effect of LY on hepatoma 3924A cells as a function of drug concentration and incubation time and the phase-dependent toxicity were investigated first, and then the combination of the two drugs was tested.

## MATERIALS AND METHODS

*Cell culture.*—Hepatoma 3924A cell line was grown in monolayer cultures in McCoy's 5A medium with 10% fetal calf serum (FCS) (Grand Island Biological Co, NY) and incubated in 5% $CO_2$:95% air. To keep the cells in logarithmic growth, they were subcultured three times a week. For experiments on exponentially growing populations, $5 \times 10^5$ hepatoma 3924A cells were seeded into 25-$cm^2$ plastic flasks and incubated for 48 hrs. For studies on plateau-phase cells, cultures were incubated for 96 hrs without changing the medium. Experimental details, definitions, and cytogenetic characteristics of plateau and exponential phases were reported elsewhere (13).

*Cell synchrony.*—Synchronized cell populations were used to study the cell cycle phase specificity of the drug. Synchrony was achieved by mitotic selection (16,17). Mitotic index was 90% as determined by counting metaphases after orcein staining in a hemocytometer. The length of the phases of the cell cycle was determined as reported previously (13). Current results indicated that the duration of each phase was: $G_1$, 7 hrs; S, 4.5 hrs; and $G_2$, 2.5 hrs. The average cell cycle time was 14 hrs.

*Dose-response experiments.*—Exponential- or plateau-phase cells were treated with different concentrations of LY. Cultures were exposed to LY for 1 or 2 hrs, then the drug was removed and flasks were rinsed three times with phosphate-buffered saline (PBS) containing (g/liter): KCl, 0.20; $KH_2PO_4$, 0.20; NaCl, 8.00; and $Na_2HPO_4 \cdot 7H_2O$, 2.16. The cells were then trypsinized, counted electronically (Coulter Electronics, Inc, Hialeah, FL), and seeded into culture flasks (500 cells/25 $cm^2$). The pH was adjusted with 5% $CO_2$ in air. After 7 days' incubation the medium was removed and the cells were stained with a saturated solution of crystal violet in 0.85% sodium chloride (13). Colonies containing > 50 cells were counted and results were expressed as percent of colonies of untreated cells. To avoid growth stimulation, plateau-phase cells were treated by adding the drugs (prepared in PBS) to the exhausted medium for 1 or 2 hrs; cells were then trypsinized and plated as described above. Long-term experiments were performed with proliferating cultures. Five hundred cells were seeded into the flasks and incubated with the drug for 7 days. Colonies were stained and counted as above. The plating efficiencies of untreated control cultures of exponentially growing cells, plateau-phase cells, and mitotically selected cells were 76%, 72%, and 50%, respectively. Mean values, standard errors, and statistical evaluation by the t test were calculated for each dose or time point. Differences between mean values yielding a probability of < 5% were considered as significant.

*Drugs.*—LY was obtained through the G. Richter Co, Budapest, Hungary. PF was donated by Eli Lilly and Co, Indianapolis, IN. Both lyophilized drugs were stored at 4°C, dissolved in sterile PBS just before use, and immediately added to the cell culture.

## RESULTS AND DISCUSSION

### Dose-Response Action of LY

Cultures in exponential or plateau phase were exposed to different drug concentrations for 1 or 2 hrs. The survival curves of cells exposed to increasing concentrations of LY for 1 or 2 hrs were of the threshold exponential type (fig 1). After a moderately large shoulder region ($D_q$ = 6.3 μM, 1 hr; $D_q$ = 1 μM, 2 hrs), survival decreased exponentially as a function of increasing concentration ($D_o$ = 3.0 μM, 1 hr; $D_o$ = 1 μM, 2 hrs).[7] Dose-response curves showed similar lethal effects on both exponential- and plateau-phase cells. This points to the possible usefulness of this drug in combination with a phase-specific agent, eg, PF.

To examine the length of biologic activity of the drug, proliferating cells were incubated with 1 μM

[7] $D_q$ = quasi-threshold dose equal to the intercept with the abscissa of the exponential part of survival curve. $D_o$ = mean lethal dose equal to the concentration required to reduce survival by 63% on the exponential part of survival curve.

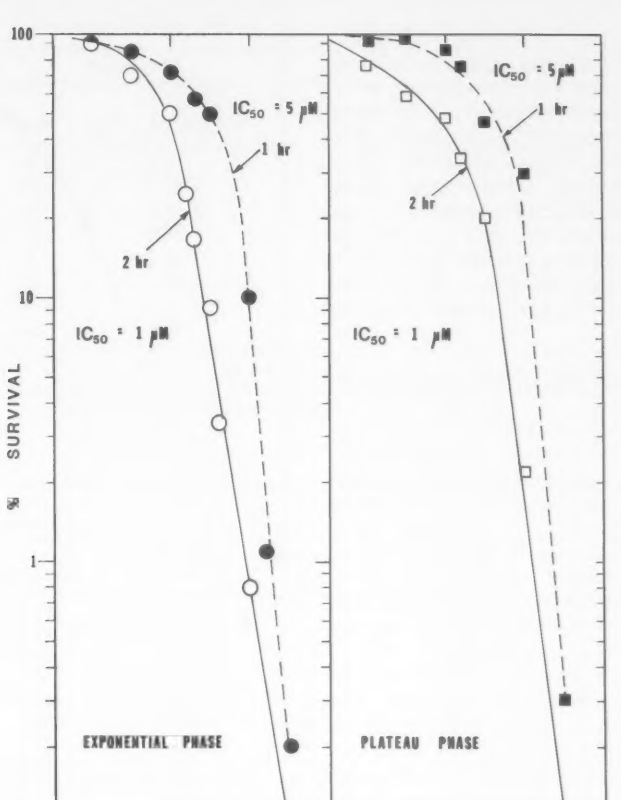


FIGURE 1.—Survival of hepatoma 3924A cells exposed to increasing concentrations of LY in exponential and plateau phases. Cells were treated for 1 or 2 hrs with the drug; after 7 days of incubation, colonies were counted and counts were expressed as % of colonies of untreated cells. Each point is the average of triplicate samples of 3 experiments.

LY up to a 24-hr period. At different intervals, cells were plated for colony formation. The cell-killing activity of LY was time-dependent (fig 2).

Long-term experiments were conducted on proliferating cells that were exposed to LY for 7 days and evaluated by colony counts. The threshold exponential type dose-response survival curve (fig 3) showed a minimal effective dose of 0.005 μM ($D_o$ = 0.014 μM and $D_q$ = 0.01 μM). On the basis of the combined data of figures 2 and 3 and table 1 (*see* below), it may be assumed that the time-dependent accumulation of the intact drug by the hepatoma cells is an important determinant of the extent of cytotoxicity of LY since low concentrations in long-term incubation were as effective as high concentrations in short-term exposure. It is important that low drug concentrations were biologically active in the prolonged treatment, since this may be relevant to clinical trials.

## Cytotoxicity of LY in the Cell Cycle

On the basis of the dose-response curves of exponentially growing cells (1 and 2 hrs of incubation) a cycle specificity of LY was expected. To elucidate the cytotoxic action of LY at different phases of the hepatoma cell cycle, synchronized cultures were

TABLE 1.—Stability of biologic activity of LY in different solutions

| Preincubation time of LY in solutions at 37°C (hrs) | Concentration of LY (μM) | % survival* | |
|---|---|---|---|
| | | McCoy's 5A + FCS | PBS† |
| 0 | 1.0 | 8.0 | 0 |
| | 0.02 | 84.0 | 44 |
| 2 | 1.0 | 9.0 | 0 |
| | 0.02 | 86.0 | 52 |
| 24 | 1.0 | 50.0 | 0 |
| | 0.02 | 67.0 | 44 |
| 48 | 1.0 | 74.7 | 0 |
| | 0.02 | 80.0 | 45 |

*Proliferating cells were exposed to LY for 7 days.
†All values are significantly lower than those for LY preincubated in complete medium ($P < 0.05$). Mean values of triplicate determinations are given.

TABLE 2.—Effect of 2-hr exposure to LY and PF in hepatoma 3924A cells

| Cell phase | LY | | PF | | % survival with LY + PF | |
|---|---|---|---|---|---|---|
| | Concentration (μM) | Survival (%) | Concentration (μM) | Survival (%) | Predicted* | Observed |
| Exponential | 0.1 | 92 | 10 | 80 | 74.0 | 88.0 |
| | 0.5 | 69 | 10 | 80 | 55.0 | 19.2† |
| | 1.0 | 50 | 20 | 50 | 25.0 | 4.6† |
| | 2.0 | 25 | 50 | 29 | 7.2 | 0.0† |
| Plateau | 0.1 | 75 | 100 | 80 | 60.0 | 81.0 |
| | 1.0 | 48 | 100 | 80 | 38.4 | 48.0 |
| | 2.0 | 34 | 100 | 80 | 27.1 | 34.0 |
| | 5.0 | 20 | 100 | 80 | 16.0 | 7.2† |

*Predicted survival = (mean % of LY survival × mean % of PF survival)/100.
†Significantly lower survival than predicted ($P < 0.05$). Mean values of triplicate determinations are given.

TABLE 3.—Effect of 7-day exposure to LY and PF in hepatoma 3924A cells

| Cell phase | LY | | PF | | % survival with LY + PF | |
|---|---|---|---|---|---|---|
| | Concentration (μM) | Survival (%) | Concentration (μM) | Survival (%) | Predicted* | Observed† |
| Exponential | 0.01 | 80 | 0.08 | 52 | 41.6 | 25.0 |
| | 0.01 | 80 | 0.1 | 36 | 28.7 | 18.0 |
| | 0.02 | 41 | 0.1 | 36 | 14.7 | 3.8 |
| | 0.02 | 41 | 0.2 | 13 | 5.3 | 1.1 |

*Predicted values were calculated as in table 2.
†All values indicated significant potentiation ($P < 0.05$). Mean values of triplicate determinations are given.

tested for 1 hr with 0.1 and 5 μM of the drug at various times of the cycle (fig 4). Similar treatment killed 10% and 50% of the cells in the asynchronous population. Cell age-dependent survival revealed fluctuations in sensitivity. The most sensitive part of the cell cycle was mid $G_1$ and then early S phase; the effect was more pronounced when the higher concentration of LY was used (5 μM). At the beginning of $G_1$ phase, 40% of the cells were killed with 0.1 μM LY, whereas 5 μM LY resulted in a 99% cell kill. The high sensitivity to LY was also seen during the entire $G_1$ phase and in early and mid S phase. In the mitotic phase, about 94% of the hepatoma cell population was killed. Late S and $G_2$ phases were relatively resistant to LY. Inhibition of cell growth in $G_1$ may be connected with the action

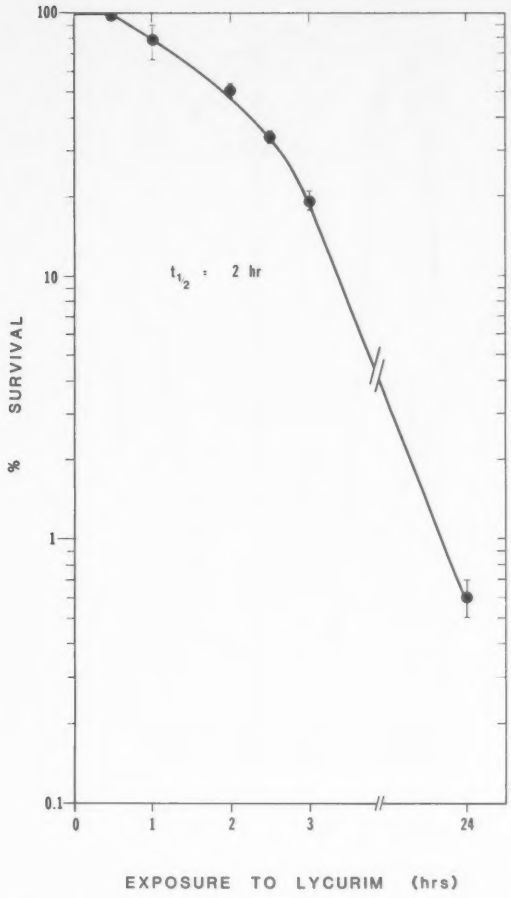


FIGURE 2.—Survival of proliferating hepatoma 3924A cells exposed to 1 μM LY for increasing periods of time. Results were evaluated as in fig 1. Each point is the average of triplicate samples of 3 experiments.

of LY on cell replication. LY inhibited the biosynthesis of purine bases, a process that occurs in $G_1$ phase (4). The strong inhibition of cell growth in S phase could result from the inhibition of DNA synthesis (4).

## Stability of the Biologic Activity of LY in Different Solutions

It was relevant to examine the stability of LY under cell culture conditions because of the length of some of the experiments. The drug was dissolved and preincubated at 37°C in complete medium (which contained FCS) and in PBS (in the absence of FCS) for various periods of time; it was then added to proliferating cells, and after 7 days the percent survival was measured by colony counts. Table 1 shows about 30%–40% higher survival when 0.02 μM LY was dissolved and preincubated in complete medium, indicating that the drug was bound to proteins or degraded in the serum. This process was time-dependent when LY was used in higher concentration (1 μM). LY binding to proteins in media reduced the amount of drug available for its cytotoxic action. For these reasons LY was always dissolved in PBS as outlined in the Materials and Methods section.

## Combination Treatment With LY and PF

To test the hypothesis that LY together with PF might potentiate the killing of hepatoma 3924A cells, exponential- and plateau-phase cells were exposed to different concentrations of both drugs for 1 and 2 hrs and for 7 days. The predicted survival and possible synergism were calculated according to Webb (18) (table 2). Mean cell survival values by corresponding doses of the single agents were used for calculating predicted survival.

When exponential- and plateau-phase cells were treated for 1 hr with LY and PF, only summation was observed. When hepatoma cells were treated for 2 hrs with the drugs, synergistic interaction was observed in exponentially growing cells at LY concentrations of 0.5–2.0 μM and PF concentrations of 10–50 μM. In plateau-phase cells, only higher drug concentrations (eg, 5.0 μM LY and 100 μM PF) yielded a synergistic response (table 2).

Seven days of treatment of proliferating hepatoma cells yielded synergistic killing action with low concentrations of LY (0.01–0.02 μM) and PF (0.08–0.2 μM) (table 3). We also observed that the current hepatoma 3924A cell line was more sensitive to PF than the line studied earlier (13). When LY and PF were given in combination at their respective IC50 concentrations (0.016 and 0.085 μM), the predicted survival was 25%, whereas the observed survival was 5.9% (data not shown). These results suggest that the synergistic activity of LY and PF was primarily time-dependent and secondarily concentration-dependent. When drugs were used in sequential treatment of exponentially growing hepatoma cells (first LY and then PF was added), no synergistic action was observed.

## Postulated Mechanism of Synergistic Action of LY and PF

It has been postulated that anticancer chemotherapy might be directed most effectively against

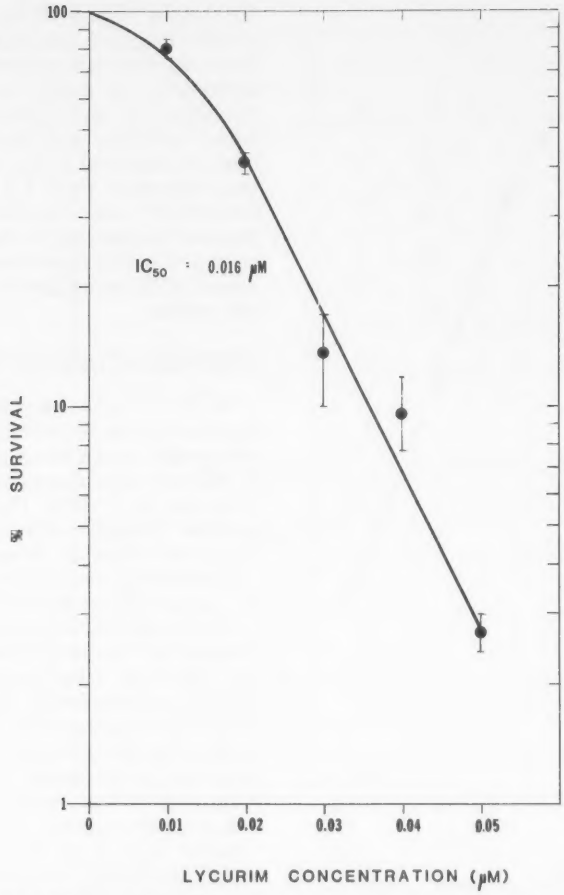


FIGURE 3.—Survival of proliferating hepatoma 3924A cells exposed to increasing concentrations of LY for 7 days. Cells were seeded (500/flask) and supplemented with the drug for 7 days. Colonies were counted and a dose-response curve was made as in fig 1. Each point is the average of triplicate samples of 3 experiments.

the activities of enzymes that are markedly increased in tumors (19,20). PF was selected for drug combination with LY because in hepatoma adenosine kinase activity was retained (21) and orotidine-5′-monophosphate decarboxylase activity, the target of PF inhibitory action of de novo uridine-5′-monophosphate synthesis (6), was markedly elevated (14,15,22,23). As therapeutic effectiveness of PF depended on the growth fraction of the cells (13), the effect of LY, which also killed resting cells, was tested. LY, as an alkylating agent, would damage the DNA molecule, binding with purines (24). There is also a new observation that LY inhibits transaldolase activity.[6] This might decrease ribose-5-phosphate concentration, possibly leading to a lowered tissue phosphoribosylpyrophosphate content. Furthermore, as the activity of hypoxanthine-guanine phosphoribosyltransferase could also be limited, the action of LY might inhibit the salvage biosynthesis of purines. The report that LY inhibited the biosynthesis of purines (4) is in line with these postulates. Thus, the cytologic observations suggest biochemical mechanisms for exploration and the potential usefulness of LY and PF in combination chemotherapy in vivo for hepatoma 3924A. Such experiments are in progress in this laboratory.

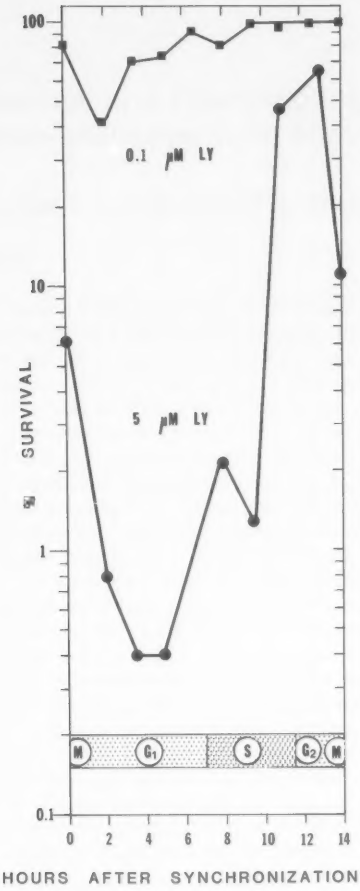


FIGURE 4.—Sensitivity of synchronous hepatoma 3924A cells to 0.1 and 5 μM LY. Synchronized cells (1000/flask) were exposed to the drug at regular intervals after mitotic selection. Drug was removed after 1 hr and the cells were washed with PBS. For each time point the synchronized untreated controls were also plated. Colonies were counted, and results were evaluated as described in the legend for fig 1. Each point is the average of triplicate samples of 2 experiments.

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# Biochemical and Pharmacologic Properties of a New Folate Analog, 10-Deaza-aminopterin, in Mice [1,2]

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## SUMMARY

**Single-dose LD50 for 10-deaza-aminopterin in mice was 236 mg/kg compared to 28 and 386 mg/kg, respectively, for aminopterin and methotrexate. Multiple-dose LD10 for 10-deaza-aminopterin was 15 mg/kg (every 2 days × 5) compared to 0.4 and 18 mg/kg, respectively, for aminopterin and methotrexate. The rate of clearance of 10-deaza-aminopterin and methotrexate from mouse plasma was essentially the same following sc administration of 12–48 mg/kg. The clearance kinetics following oral administration of 48 mg/kg of each agent was also similar. However, following oral administration of 6 mg/kg, plasma levels were twofold higher for 10-deaza-aminopterin. Inhibition of tumor cell dihydrofolate reductase was essentially the same for 10-deaza-aminopterin, aminopterin, and methotrexate. The differences in lethal potency of 10-deaza-aminopterin and methotrexate correlated with the persistence of exchangeable levels of each agent in mouse small intestine. The differences in antitumor effects in L1210 leukemia and Sarcoma 180 models shown for 10-deaza-aminopterin and methotrexate during earlier studies also correlated with the persistence of exchangeable levels of each agent in the tumor cell population after sc administration in vivo. The extent of persistence of each antifol in tumor or small intestine could be accounted for by differences in membrane transport, specifically the apparent influx $K_m$, which is a measure of the affinity of the transport system in each cell type for the three analogs. These differences also correlated with the relative inhibitory potency of the three analogs against L1210 leukemia cells in culture. The greater antitumor efficacy against L1210 leukemia and Sarcoma 180 tumors in vivo observed earlier with 10-deaza-aminopterin compared to methotrexate and aminopterin appears to reflect a more favorable relative interaction of the new analog with the respective transport system in tumor compared with normal proliferative tissue in small intestine.**

[Cancer Treat Rep 66:351–358, 1982]

[1]Received Mar 10, 1981; revised July 1, 1981; accepted Aug 19, 1981.

[2]Supported in part by Public Health Service grants CA-08748, CA-18856, and CA-22764 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and grant CH-26 from the American Cancer Society.

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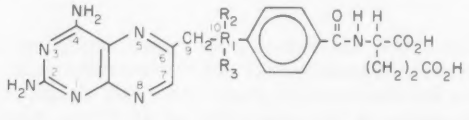
With few exceptions [most prominently acute lymphocytic leukemia (1) and choriocarcinoma (2)], most human cancers (2,3) are either only marginally responsive or not susceptible at all to the folate analog methotrexate. In an effort to develop new analogs that are more effective against a broader range of human cancers, we have sought information on the underlying basis for relative responsiveness, toxicity, and selective action of the general category of folate analogs. Some correlation between uptake of methotrexate and relative re-

sponsiveness of a group of murine leukemias was reported in an earlier study (4) done elsewhere. Evidence gathered more recently in our own laboratory at both biochemical (5–7) and pharmacologic (7–9) levels strongly suggests that, at least in murine tumor models, the degree of selective antitumor action of folate analogs is, to a large extent, determined at the level of membrane transport. In the case of L1210 leukemia and other lymphoid murine tumor models, folate analogs exhibit (6,7) a relatively high degree of selective antitumor action. This was associated (6,7) with greater ability of the tumor cells for carrier-mediated accumulation of drug compared to that seen in drug-limiting normal proliferative tissue, in this case, crypt epithelium in the small intestine. Alkylation at the $N^{10}$ position further increased this differential in uptake by affecting entry much more in epithelial cells than in tumor cells. Similar differences in specificity for transport involving the $N^{10}$ position also appear to explain (10) lower responsiveness of other murine tumors. For these reasons our search for new agents has focused initially on studies of analogs altered at the $N^{10}$ position.

In an earlier report (11), we presented an evaluation of the antitumor activity of a new analog, 10-deaza-aminopterin, relative to both methotrexate and aminopterin, against a variety of ascitic and solid murine tumors, some of which are predictive of responsiveness in human cancer. The data presented clearly documented the superiority (twofold to threefold greater antitumor effects) of 10-deaza-aminopterin over the other two analogs in three nonlymphoid tumors: Sarcoma 180, Ehrlich carcinoma, and the Taper liver tumor. Smaller differences, but still favoring the new analogs, were obtained in the L1210 and P815 leukemias. In the present report we now consider some relevant aspects of the toxicology and pharmacology of this new analog with respect to methotrexate and aminopterin. We also present data obtained from a comparative study of these analogs as inhibitors of dihydrofolate reductase. Other studies are also reported on the membrane transport of these analogs in vitro in L1210 leukemia, Ehrlich carcinoma, Sarcoma 180, and isolated murine intestinal epithelial cells. The results of these latter studies document the difference in membrane transport of these three analogs among the three tumor cell types and in epithelial cells which correlated with the pharmacokinetics for each analog in these tissues. Overall, our results appear to explain the relatively high level of response of these tumors to the new analog.



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| aminopterin | N | H | - |
| methotrexate | N | $CH_3$ | - |
| 10-deaza-aminopterin | C | H | H |

FIGURE 1.—Structural relationship between aminopterin, 10-deaza-aminopterin, and methotrexate.

## MATERIALS AND METHODS

Only a brief outline of experimental methods will be presented here, since very detailed descriptions have already been given in several previous reports (5–9) from this laboratory. Harvesting of tumor cells from the peritoneal cavity and maintenance of the cell lines in female $BD2F_1$ mice (obtained from Sprague-Dawley, Inc, Madison, WI) have been described earlier (5–9). Epithelial cells were isolated (6,7,11) by hyaluronidase treatment of mucosa in everted small intestine. This procedure provides a twofold to threefold purification of crypt cells, so that the final suspension consists of approximately equal amounts of crypt and mature columnar (absorptive) cells. Initial influx and efflux measurements were made (5–9) in a buffered-salts medium plus 10 mM glucose (pH 7.4). A double-reciprocal plot of the influx data (vel/[drug]) was constructed to obtain values for maximum velocity ($V_{max}$) and the apparent Michaelis constant ($K_m$). Values for influx $K_i$ were derived from similar data measuring the influx of [$^3$H]methotrexate in the presence and absence of the competing analog and using the following calculation: $K_i = [I]/(K_p/K_m - 1)$, where $K_p$ is the apparent $K_m$ in the presence of inhibitor. Efflux of drug at 37°C was expressed as values for the first-order rate constant derived (12) during measurement of the initial time-course for exit.

During pharmacokinetic experiments, blood was obtained, tumor cells were harvested from the peritoneal cavity, and the small intestine was removed and washed after sc administration of drug (2–4 days after ip implanting $10^6$ cells) as already described (5–9,12). The drug content in cell extracts and serum was determined by spectrophotometric assay measuring inhibition of a microbial dihydrofolate reductase (12,13). The dihydrofolate reductase content in cell extract and the inhibition of this enzyme by various folate analogs were determined by a titration assay described elsewhere (13). All three folate analogs (*see* fig 1 for structural formulas) were prepared as the sodium salt in neutral aqueous solution. The synthesis of 10-deaza-aminopterin has been described earlier (14). Samples of methotrexate and

TABLE 1.—Lethal toxicity of folate analogs in female $BD2F_1$ mice

| Analog | Toxicity* Single-dose LD50 (mg/kg) | Multiple-dose† LD10 (mg/kg) |
|---|---|---|
| Aminopterin | 28 | 0.40 |
| 10-Deaza-aminopterin | 236 | 15 |
| Methotrexate | 380 | 18 |

*Average of 2–4 experiments employing 5 mice/group receiving different dose of each analog. Value for LD was obtained from a plot of % deaths vs log dose.

†Analog given as 1 dose every other day × 5.

aminopterin used during these studies were provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Samples of 10-deaza-aminopterin (> 98% pure) were synthesized (14) by one of us (Dr. J. I. DeGraw).

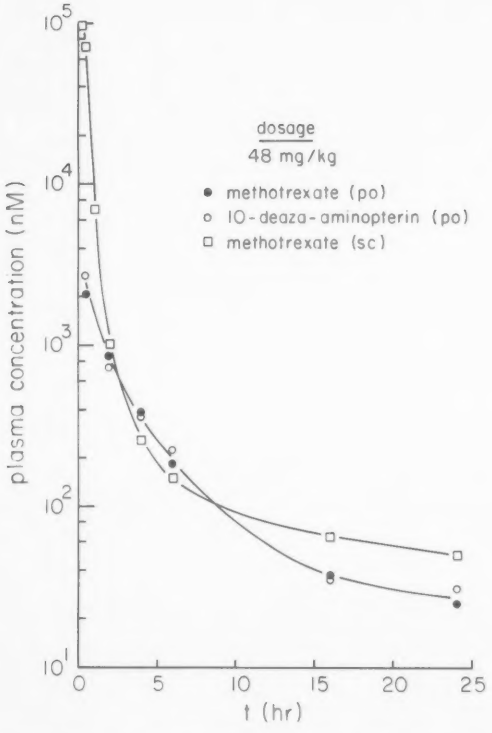



FIGURE 2.—Clearance of methotrexate and 10-deaza-aminopterin in mouse plasma after oral or sc administration of 48 mg/kg. Each point is an average of values obtained from 2 experiments, with 3 mice/point. SEM did not exceed ±15%.

## RESULTS AND DISCUSSION

### Relative Toxicity of 10-Deaza-aminopterin

Single- and multiple-dose lethality of 10-deaza-aminopterin was compared to that of aminopterin and methotrexate in $BD2F_1$ female mice. The results are given in table 1. A markedly greater lethal potency of aminopterin relative to methotrexate was observed in the present study, which confirms results well-documented in earlier studies from this laboratory (15,16) and elsewhere (17,18). The data also show that when given as either a single dose or in a multiple-dose schedule, 10-deaza-aminopterin is similar in lethality to methotrexate, although slightly more potent.

### Plasma and Tissue Pharmacokinetics of 10-Deaza-aminopterin Compared to Aminopterin and Methotrexate

The clearance of 10-deaza-aminopterin and methotrexate from mouse plasma was determined for a dose of 12 and 48 mg/kg sc. The lower dose is within the maximum tolerated dose range for both 10-deaza-aminopterin and methotrexate when given on a multiple-dose schedule (table 1). The results showed (data not given) that the time-course for clearance of the two agents following this route of administration was essentially the same. A prior report (15) from this laboratory has already provided other data showing similar clearance characteristics of methotrexate and aminopterin parenterally administered to mice at somewhat lower doses. At the doses shown here, maximum levels of each analog in plasma occurred by 15 mins. These concentrations were 2–3 and 8–10 × $10^{-5}$ M following 12 and 48 mg/kg, respectively, of either methotrexate or 10-deaza-aminopterin. The terminal elimination phase (16) for both analogs in these mice was observed approximately 10 hrs after administration. Following oral administration of 10-deaza-aminopterin and methotrexate at a dose of 48 mg/kg, the clearance characteristics in plasma were also similar for both agents (fig 2). However, by comparison with the same dose given sc (fig 2), differences between the routes of administration were observed in the overall kinetics of clearance. After oral administration of 6 mg/kg of each agent, which is in the therapeutic range, plasma levels of 10-deaza-aminopterin (fig 3) were twofold higher than levels of methotrexate. This result is suggestive of greater bioavailability of 10-deaza-aminopterin at lower doses and serves to explain the twofold greater therapeutic effect of this new analog compared to methotrexate (11) against the L1210 murine leukemia, following oral administration.

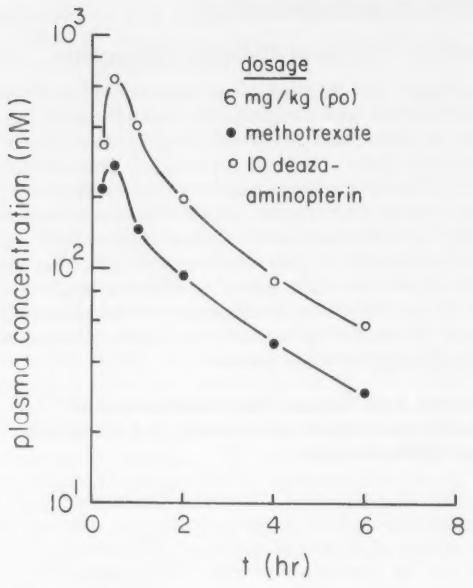



FIGURE 3.—Clearance of methotrexate and 10-deaza-aminopterin in mouse plasma after oral administration of 6 mg/kg. Each point is an average of values obtained from 2 experiments, with 3 mice/point. SEM did not exceed ±16%.

A positive correlation between the persistence of exchangeable levels of different antifolates in mouse small intestine, the drug-limiting organ, and relative toxicity has been documented in earlier reports (7,9,17) from this laboratory. Exchangeable levels of drug refer to that fraction of total intracellular drug which is not bound to dihydrofolate reductase. This fraction of drug was determined (7) by subtracting the dihydrofolate reductase binding equivalence for each tissue from the value of total intracellular drug determined at each interval. Greater persistence of exchangeable levels of antifolate also occurred (8,9) in responsive tumors compared with small intestine tissue which appeared to explain the selective antitumor action of these agents in mice. Moreover, a correlation between antifolate retention above the dihydrofolate reductase binding level and relative responsiveness of a number of different murine tumors has also been documented by studies (7,9) in this laboratory. We sought to obtain similar data in the case of 10-deaza-aminopterin which might explain the increased antitumor efficacy of this agent against these same tumors in comparison to methotrexate.

The results of a series of experiments with the Sarcoma 180 model, a murine tumor relatively insensitive to methotrexate but highly responsive to 10-deaza-aminopterin (11), are given in figure 4. In these experiments, the persistence of exchangeable levels of 10-deaza-aminopterin and methotrexate with time following sc administration of either 3 or 12 mg/kg to tumor-bearing mice was determined for the small intestine and the tumor cell population in the peritoneal cavity. The initial time-course for accumulation in each tissue did not differ appreciably between the two analogs (data not shown). In the small intestine the period during which exchangeable levels of 10-deaza-aminopterin persisted following doses of either 3 or 12 mg/kg was only slightly greater than that seen for methotrexate. This result is in agreement with the relatively small difference in lethal toxicity of the two agents (table 1) when given in a multiple-dose schedule. In Sarcoma 180 cells, however, the persistence of these two agents is considerably different. Following doses of both 3 and 12 mg/kg, exchangeable levels of 10-deaza-aminopterin were maintained substantially longer than levels of methotrexate. This difference in the persistence of the two agents in tumor cells compared to that seen in the small intestine, which by itself is suggestive of a greater antitumor effect of 10-deaza-aminopterin, does, in fact, correlate with the results (11) of our therapy experiments with this tumor model. In a related experiment, the persistence of 10-deaza-aminopterin and methotrexate following an sc dose of 12 mg/kg was compared (fig 5) for L1210 tumor cells in leukemic mice. In L1210 cells the persistence of both analogs was appreciably greater than that seen in small intestine. The results pertaining to the small intestine are not shown since they were the same as those already shown in figure 4. The persistence of 10-deaza-aminopterin in the leukemia cells, however, was only slightly greater than that of methotrexate. Also, the persistence of these two agents in L1210 cells was similar to that seen for 10-deaza-aminopterin in Sarcoma 180 cells. Both findings are also consistent with the results obtained (11) during therapy experiments showing a similarly high level of therapeutic efficacy of both agents against L1210 leukemia in mice, but a somewhat greater effect of 10-deaza-aminopterin.

## Inhibition of Dihydrofolate Reductase by 10-Deaza-aminopterin and Methotrexate

Methotrexate, aminopterin, and 10-deaza-aminopterin were similar in their inhibition of dihydrofolate reductase in L1210 cells. This is seen from the data presented in figure 6. At pH 6.2, all three agents inhibited this enzyme almost stoichiometrically. However, at pH 7.3 and 8.0, 10-deaza-aminopterin was a somewhat better inhibitor than either

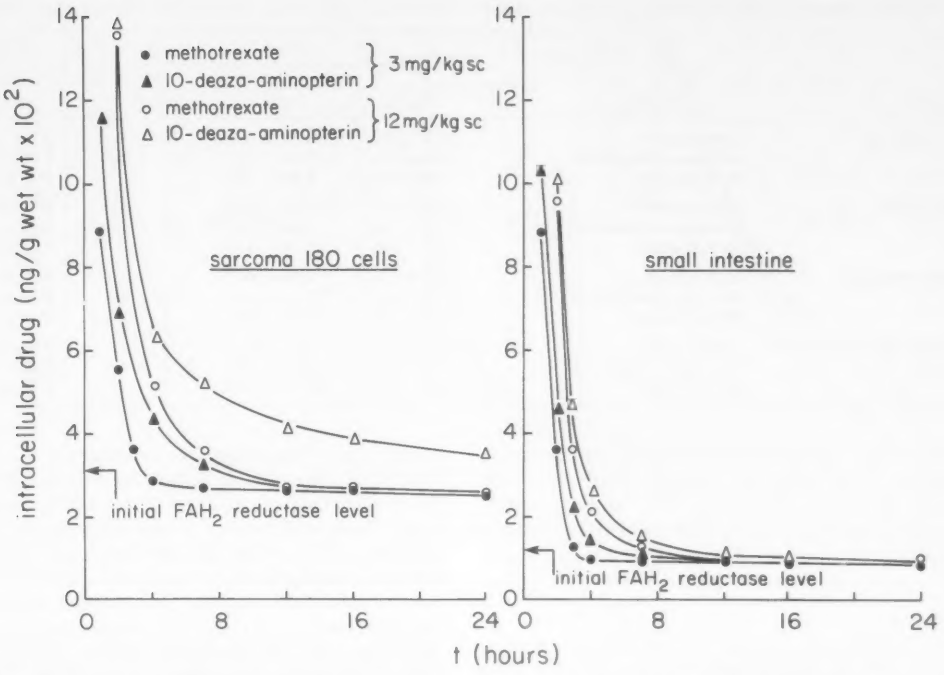



FIGURE 4.—Retention of methotrexate and 10-deaza-aminopterin in Sarcoma 180 cells and mouse small intestine after sc administration of 3 or 12 mg/kg to tumor-bearing mice. Values shown for each dose are averages of 2–4 individual determinations at the indicated times, obtained from 3 experiments done on different days.

of the other analogs at concentrations which exceed the molar equivalence in enzyme concentration. Estimated values for $K_i$ (24) at pH 7.3 were $6 \times 10^{-12}$ M for methotrexate, $4 \times 10^{-12}$ M for aminopterin, and $2 \times 10^{-12}$ M for 10-deaza-aminopterin. Results similar to those seen for the L1210 cell enzyme were also obtained (data not shown) with enzyme derived from Sarcoma 180 cells.

## Membrane Transport of 10-Deaza-aminopterin and Methotrexate

Properties of the folate analog transport system in murine tumor cells have been described in reports (5–7) from this laboratory and elsewhere (19–21). The system in isolated epithelial cells from mouse small intestine has also been described (6,7,22), but to a lesser extent. The tumor cell systems also transport natural folate derivatives and are qualitatively similar, exhibiting Michaelis-Menten saturation kinetics, competition among structural analogs, and high temperature-dependence. The system in intestinal epithelial cells, while less defined, does appear to exhibit similar kinetic features.

During the present studies transport of aminopterin, 10-deaza-aminopterin, and methotrexate were compared in L1210 leukemia, Sarcoma 180, Ehrlich carcinoma, and intestinal epithelial cells to elucidate differences among the three agents which would allow a clearer interpretation of the previously reported therapy data (10) and the pharmacokinetic data described in the present report. A summary of the most relevant results is presented in table 2. The individual kinetic values were derived during experiments (*see* Materials and Methods) measuring the time-course for initial influx and efflux. Since the time-courses for these rate measurements were similar to those already reported (5–9) from this laboratory, they will not be given here. It can be seen that values derived for influx capacity ($V_{max}$) and efflux (first-order rate constant) varied somewhat among the different tumor cells. Larger differences in these parameters were seen in the case of the isolated intestinal epithelium. However, in each tissue, essentially the same values were derived for the three analogs studied. In contrast to these results, major differences among these cell types were observed for values of influx saturability

TABLE 2.—Kinetic analysis of mediated transport of folate analogs by normal mouse proliferative cells and tumor cells

| Cell type | Analog | Influx* | | | Efflux* |
|---|---|---|---|---|---|
| | | $V_{max}$† | $K_m$ (μM) | $K_i$‡ (μM) | K ($min^{-1}$) |
| L1210 leukemia | Aminopterin | 3.33 ± 0.5 | 1.11 ± 0.2 | | 0.21 ± 0.021 |
| | 10-Deaza-aminopterin | 3.26 ± 0.4 | 1.13 ± 0.1 | 1.7 ± 0.2 | 0.20 ± 0.032 |
| | Methotrexate | 3.29 ± 0.3 | 3.84 ± 0.4 | | 0.19 ± 0.024 |
| Sarcoma 180 | Aminopterin | 3.62 ± 0.4 | 2.12 ± 0.2 | | 0.21 ± 0.034 |
| | 10-Deaza-aminopterin | 3.56 ± 0.5 | 1.04 ± 0.1 | 1.12 ± 0.4 | 0.22 ± 0.025 |
| | Methotrexate | 3.75 ± 0.4 | 11.41 ± 1.4 | | 0.18 ± 0.019 |
| Ehrlich carcinoma | Aminopterin | 2.42 ± 0.3 | 1.96 ± 0.3 | | 0.17 ± 0.021 |
| | 10-Deaza-aminopterin | 2.58 ± 0.2 | 1.08 ± 0.2 | 1.19 ± 0.3 | 0.16 ± 0.018 |
| | Methotrexate | 2.71 ± 0.2 | 8.63 ± 0.9 | | 0.16 ± 0.019 |
| Epithelial (small intestine) | Aminopterin | 2.81 ± 0.4 | 19.4 ± 3.7 | | 0.09 ± 0.01 |
| | 10-Deaza-aminopterin | 2.52 ± 0.3 | 90.3 ± 18.0 | 78.9 ± 19 | 0.08 ± 0.02 |
| | Methotrexate | 2.41 ± 0.3 | 270.0 ± 36.0 | | 0.08 ± 0.01 |

(apparent $K_m$). All values for influx $K_m$ for the tumor cells were lower than the corresponding values for isolated intestinal epithelium. This implied higher influx of the analog in tumor cells than in the isolated epithelial cells at external concentrations below saturation. For methotrexate, values for $K_m$ varied from 3.34 μM in L1210 leukemia cells to 8.63 μM in Ehrlich carcinoma cells and 11.4 μM in Sarcoma 180 cells. These differences, which were reported from our laboratory earlier (5), correlate with the relative responsiveness of these tumor cells during therapy in vivo. In L1210 cells, the $K_m$ value for aminopterin was extremely low (1.11 μM), approximately threefold lower than that for methotrexate; it was slightly higher in Ehrlich carcinoma (1.96 μM) and Sarcoma 180 (2.12 μM) cells. For 10-deaza-aminopterin the value for $K_m$ (1.0–1.2 μM) in the three tumor cell types was uniformly low. As indicated by the similarity of values for $K_m$ and $K_i$, the latter obtained during competition experiments with [$^3$H]methotrexate or [$^3$H]aminopterin (data not shown), this new analog and other analogs share the same carrier system for influx. In isolated epithelial cells the $K_m$ values for the three analogs varied widely: aminopterin (19.4 μM), 10-deaza-aminopterin (90.3 μM), and methotrexate (270.0 μM). The values for aminopterin and methotrexate were substantially higher than values reported earlier (6) from our laboratory. However, the results essentially duplicate the relative differences in the values derived in each study. The differences in the values derived in each study appear to be related to the fact that animals with

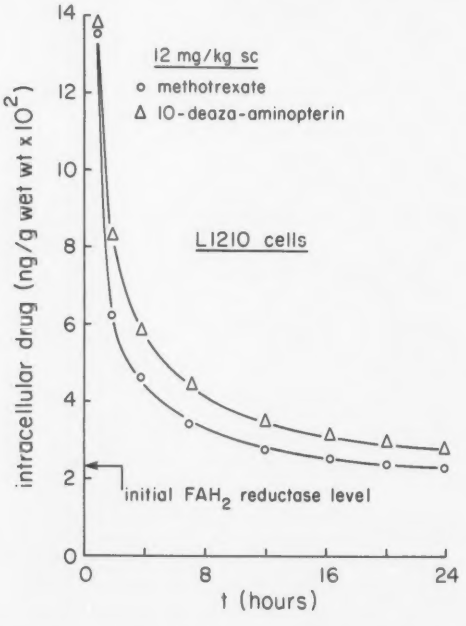



FIGURE 5.—Retention of methotrexate and 10-deaza-aminopterin in L1210 leukemia cells after sc administration of 12 mg/kg to leukemic mice. Values shown for each dose are averages of 2–4 individual determinations at the indicated times, obtained from 3 experiments done on different days. SEM did not exceed ±15%.

TABLE 3.—Inhibition of L1210 cell growth in culture by various folate analogs

| Analog | No. of cultures | IC50* (nM) |
|---|---|---|
| Aminopterin | 3 | 0.61 ± 0.07 |
| 10-Deaza-aminopterin | 4 | 0.63 ± 0.35 |
| Methotrexate | 4 | 2.71 ± 0.09 |

*Concentration required for 50% inhibition of growth ± SE.

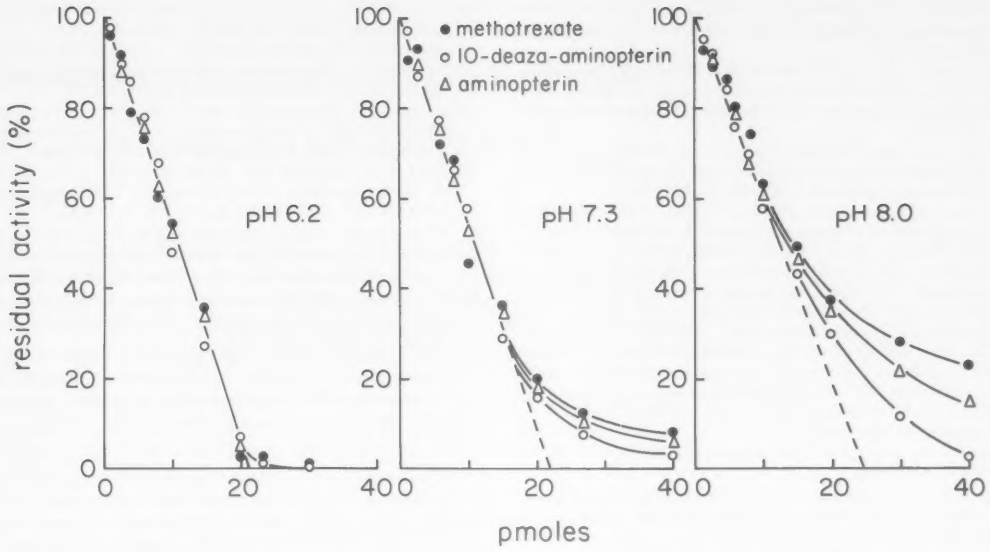



FIGURE 6.—Inhibition of dihydrofolate reductase from L1210 leukemia cells by methotrexate, aminopterin, and 10-deaza-aminopterin. Values given are an average from 3 experiments done on different days. SEM did not exceed ±13%.

greater tolerance to antifolates (23) were used in the more recent studies. In the earlier (6) and current studies the differences found in the values for the influx $K_m$ for methotrexate and aminopterin in tumor versus isolated intestinal epithelial cells provide an explanation (5–9) for the selective antitumor action of these agents in mice. The data obtained for each analog in isolated intestinal epithelial cells also explain (5–9,17) the greater lethal potency of aminopterin compared to methotrexate and the higher therapeutic index (11) of the latter against these murine tumors. When evaluated within the same context, the transport data derived with 10-deaza-aminopterin suggest an explanation for the greater therapeutic efficacy (11) of this new analog against both Sarcoma 180 and Ehrlich tumors which was inferred from the pharmacokinetic data. Somewhat greater retention of exchangeable levels of 10-deaza-aminopterin in L1210 leukemia cells and markedly greater retention in Sarcoma 180 cells (and probably Ehrlich carcinoma cells) compared to methotrexate can be attributed to more rapid influx of this new analog by the carrier-mediated transport system in these cells in the face of the rapid reduction in plasma level.

### Inhibition of L1210 Cell Growth in Culture by Folate Analogs

Further data on the relative antitumor potency of methotrexate, aminopterin, and 10-deaza-aminopterin were obtained during experiments measuring inhibition of L1210 cell growth in culture (table 3). In this assay, both aminopterin and 10-deaza-aminopterin were fourfold to fivefold more potent than methotrexate. These results are in general agreement with the relative potency predicted by data on dihydrofolate reductase inhibition (fig 6) and on membrane transport (table 2) of these analogs in L1210 leukemia cells.

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# Potentiation of Bleomycin Toxicity by Oxygen[1,2]

C. H. Toledo, W. E. Ross,* C. I. Hood, and E. R. Block[3,4]

## SUMMARY

**The clinical usefulness of bleomycin is limited by pulmonary toxicity. Recent clinical observations indicate that the pulmonary toxicity is potentiated in patients who are exposed to elevated but nontoxic concentrations of $O_2$. We studied the interactions of $O_2$ and bleomycin using a murine model. Mice were divided into two groups. One group was continuously exposed to 40% $O_2$ at 1 atmosphere while the other group breathed compressed room air. The elevated $O_2$ concentration alone was not toxic to animals. Mice in each group received either 40 mg/kg of bleomycin or normal saline twice a week sc. Median survival of mice receiving bleomycin was shortened from 8.24 to 4.35 weeks when the animals were maintained on 40% $O_2$ ($P = 0.0001$). We conclude that exposure to a nontoxic but elevated $O_2$ concentration can potentiate the toxic effects of bleomycin.**

[Cancer Treat Rep 66:359–362, 1982]

A major problem in the clinical use of the antitumor antibiotic bleomycin is the occurrence of dose-related pulmonary toxicity (1). Pulmonary infiltrates and fibrosis have been reported in up to 10% of all patients receiving the drug, with approximately 1% associated mortality (2). The risk of bleomycin pulmonary toxicity appears to increase with cumulative drug dose, increasing age, and prior pulmonary radiation (2). A retrospective clinical observation suggested that patients previously treated with bleomycin may unexpectedly develop pulmonary toxic effects when exposed to elevated but normally nontoxic concentrations of $O_2$ (3). The purpose of this study was to confirm whether bleomycin toxicity is potentiated by a high but otherwise nontoxic concentration of inspired $O_2$, using an experimental animal model.

## MATERIALS AND METHODS

Male NIH/Swiss mice weighing approximately 25 g were randomly divided into two groups. One group was housed in an airtight Plexiglas chamber and exposed to compressed air at 1 atmosphere of pressure and a flow rate of 5 liters/minute. A second group was housed in a similar chamber and exposed to 40% $O_2$/60% $N_2$ at 1 atmosphere and a flow rate of 5 liters/minute. This $O_2/N_2$ mixture was maintained using a Bird Oxygen Blender (Bird Corp, Palm Springs, CA), and the $O_2$ concentration was monitored by a Beckman Oxygen Analyzer (model E2). The chambers were opened approximately once a day for cage cleaning and/or injection of the mice. The humidity inside the two chambers was monitored using a hygrometer (Scientific Products) and was comparable throughout the experiment (75%–90%). Each group of animals was further subdivided to receive injections of normal saline (controls) or bleomycin (40 mg/kg) twice a week. These groups are summarized in table 1. Each group of mice was weighed at weekly intervals and the mean weight

[1]Received May 19, 1981; revised July 13, 1981; accepted July 22, 1981.

[2]Supported by a grant from the Florida Lung association and Public Health Service grant CA-00537 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Medicine (*C. H. Toledo, W. E. Ross,* and *E. R. Block*), Pharmacology (*W. E. Ross*) and Pathology (*C. I. Hood*), University of Florida College of Medicine, and Laboratory Service (*C. I. Hood*), Veterans Administration Medical Center, Gainsville.

[4]E. R. Block is an Edward Livingston Trudeau Fellow of the American Lung Association.

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TABLE 1.—Summary of treatment groups

| Group | No. of mice* | $FiO_2$† | Treatment‡ |
|---|---|---|---|
| 1 | 10 | 0.20 | Saline |
| 2 | 17 | 0.20 | Bleomycin |
| 3 | 10 | 0.40 | Saline |
| 4 | 17 | 0.40 | Bleomycin |

*Nos. do not include mice used for this histopathologic correlation.

†$FiO_2$ = fractional inspired $O_2$ concentration.

‡Bleomycin (40 mg/kg) and saline were administered sc twice a wk.

per mouse was calculated. Survival was monitored daily.

Lung histology was sequentially examined in a separate group of mice treated similarly to those in groups 1–4. These animals were used for histopathologic correlation only and were not considered in the analyses of survival. Lungs were inflated and fixed for histologic examination by reinfusion with 10% Formalin. Tissue was stained with hematoxylin and eosin, Masson trichrome, and periodic acid-Schiff stains.

## RESULTS

Mice were exposed to air or $O_2$ for up to 10 weeks. During $O_2$ exposures the $O_2$ concentration in the chamber ranged from 38% to 42%. Weight gain in control mice breathing air (group 1) was comparable to weight gain in control mice breathing 40% $O_2$ (group 3). Both groups continued to gain weight throughout the experiment (fig 1). In contrast, the mice receiving bleomycin consistently lost weight, and this effect appeared to be initially more severe in the mice breathing $O_2$ (group 4) than in those breathing air (group 2).

Survival data are shown in figure 2. No deaths were recorded in control mice exposed to air (group 1). Similarly, exposure to 40% $O_2$ alone had no effect on survival. The only death in group 3 was a result of a handling error during Week 1 of the experiment. In contrast, treatment with bleomycin re-

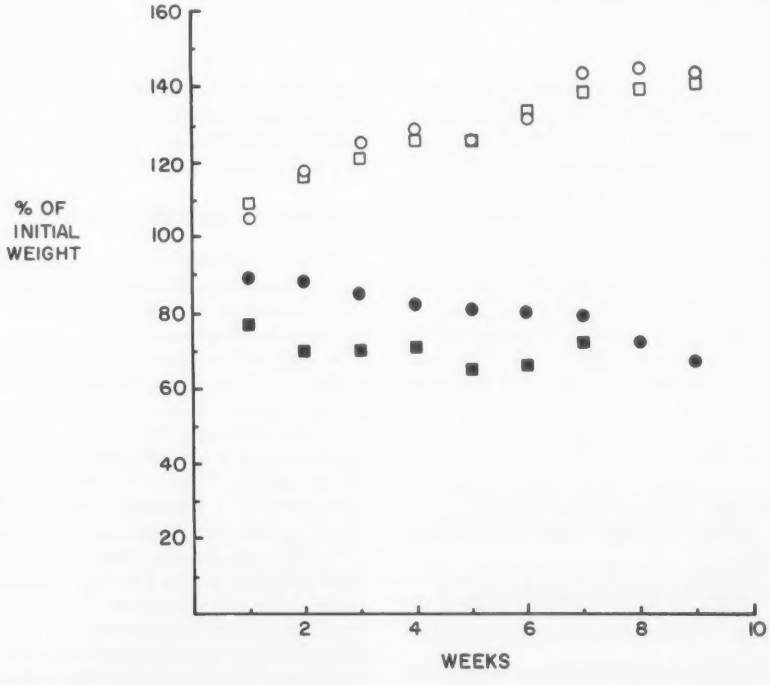


FIGURE 1.—Effect of bleomycin on body weight of mice breathing air or 40% $O_2$. Mice received saline (open symbols) or 40 mg/kg of bleomycin (closed symbols) sc while breathing air (circles) or 40% $O_2$ (squares). Mean weight per mouse in each group was calculated weekly and was compared to pretreatment values.

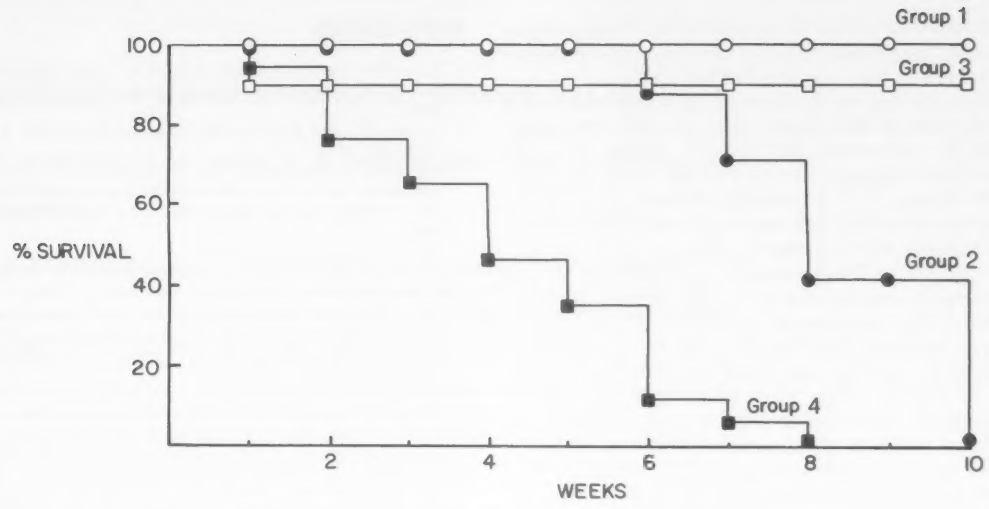


FIGURE 2.—Survival of mice receiving bleomycin in air or 40% $O_2$. *See* fig 1 for explanation of symbols.

sulted in animal mortality and this effect was greatest in the animals breathing $O_2$. The median duration of survival for the mice receiving bleomycin while exposed to air (group 2) was 8.24 weeks. The first death in this group occurred at 6 weeks, and by 10 weeks, mortality was 100%. The median duration of survival for the mice receiving bleomycin while exposed to 40% $O_2$ (group 4) was 4.35 weeks; by the end of 8 weeks, this group had 100% mortality. The difference in survival between groups 2 and 4 was highly significant ($P = 0.0001$, two-sample t test).

The lungs from 12 mice, representing all four groups, were examined at varying intervals during the study. Groups 1 and 3 remained normal throughout the 10-week observation period. All animals treated with bleomycin (groups 2 and 4) exhibited pulmonary changes consistent with the effects of bleomycin (4,5) including interstitial edema, loss of the alveolar pattern, increase of reticulin fibers, and infiltration by macrophages, lymphocytes, and plasma cells. The only differences between groups 2 and 4 were noted after Week 4. Lungs from animals simultaneously exposed to 40% $O_2$ and bleomycin exhibited more extensive alveolar destruction and collapse and fewer alveolar macrophages than those exposed to bleomycin in room air.

## DISCUSSION

This study was prompted by the retrospective observation of Goldiner et al (3) that a small group of patients who had previously received bleomycin developed postoperative adult respiratory distress syndrome when exposed to elevated but nontoxic inspired $O_2$ concentrations intraoperatively. Because of the important clinical implications of this observation, we felt that the interaction between $O_2$ and bleomycin should be examined using a controlled experimental model. Our data confirm that a nontoxic $FiO_2$ of 0.40 can cause an increase in weight loss and a significantly increased mortality in mice treated with bleomycin. Based on our histologic data, we cannot be certain at this time that the lung was the site of this interaction. While significant mortality was experienced in group 4 during the first 4 weeks of the study, in contrast to group 2, the lungs from the two groups did not differ significantly until later in the study. Others have experienced difficulty in correlating the degree of pulmonary histologic damage with survival in bleomycin-treated mice (5). Because of the consistent observation of labored breathing, cyanosis, and foaming around the nares in the days immediately before death, we believe that our animals died of respiratory failure. However, we cannot rule out the possibility that other organ systems such as bone marrow or gastrointestinal tract may become targets of bleomycin toxicity in the presence of an elevated $O_2$ concentration. Our data do not allow us to comment on the importance of concurrent versus sequential administration of $O_2$ and bleomycin in producing potentiation or on the dose-response re-

lationship for either bleomycin or $O_2$. These are obviously important issues in drawing clinical correlations, and they require further study.

Considering the mechanism by which bleomycin is thought to kill tumor cells, it is not surprising that $O_2$ potentiates its toxicity. Within the cell, bleomycin chelates ferrous iron and binds to DNA. The bleomycin-$Fe^{+2}$ complex reduces ambient $O_2$, thereby generating the superoxide and hydroxyl free radicals (6), which ultimately damage DNA. In fact, the extent of DNA damage is dependent on the ambient $O_2$ concentration (7). The accelerated mortality in the mice breathing 40% $O_2$ in the present study suggests that the mechanism of bleomycin pulmonary toxicity may share certain features with its antitumor effect. Further work is required to determine if the pulmonary toxicity is based on damage to DNA or other intracellular macromolecules such as membrane lipids, as suggested by Yamanaka et al (8). Such a distinction is important, as it may provide a means of dissociating the therapeutic effect of bleomycin from its toxic effects.

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# Noninvasive Documentation of Emesis in Cats[1,2]

L. E. McCarthy,* J. H. Fetting, J. A. Daubenspeck, and H. L. Borison[3,4]

## SUMMARY

**Chest and abdominal wall movements were assessed as objective indicators of emesis. Thirty-six vomiting episodes in one intact and four decerebrate cats were monitored either by an inductive plethysmograph or by magnetometers applied to the chest and abdomen. The plethysmographic method was found to be the more suitable of these two monitoring techniques because it produced stable, artifact-free recordings with excellent signal differentiation. The occurrence of emesis was validated by simultaneously recording thoracic central venous pressure. Unlike the intrathoracic pressure measurement, spasmodic movements of chest and abdomen did not differentiate retching from expulsion. However, rhythmic abdominal excursions recorded during vomiting were several times greater than those occurring during any other activity. Surprisingly, these movements indicated an increase in abdominal dimensions during vomiting in contrast to the reduction in size observed during sneezing and coughing. We believe that recording of abdominal movements that accompany vomiting might potentially serve as a noninvasive research tool for the study of emesis in cancer chemotherapy.**

[Cancer Treat Rep 66:363–368, 1982]

The increasing use of intensive cancer chemotherapy in recent years has accentuated the need for effective antiemetics (1,2). Development of these agents has, however, been hindered by an inability to assess objectively the emetic liability of various therapeutic regimens under clinical conditions (3).

Based on studies performed in this laboratory (4,5), we have developed an animal model for objectively quantifying emesis in unanesthetized cats (6,7). This technique records the actual thoracic pressures that effectuate vomiting. Although this method offers rigorous quantification, it requires placing a monitoring catheter in a central thoracic vein, a procedure with a small but significant risk in clinical practice.

The present work was undertaken to determine whether a noninvasive measurement of emetic activity, such as associated movements of the chest and/or abdominal walls, might serve as reliable indicators of vomiting. Our findings in the cat are sufficiently encouraging, we feel, to warrant further testing for clinical application.

## METHODS

Observations of emetic activity were made in five adult, unanesthetized cats. Four of the animals were decerebrated and one was intact. Midbrain decerebration was performed under pentobarbital anesthesia 24 hours prior to inducing emesis. Body temperature was maintained at 37°C overnight to ensure metabolism of the anesthetic.

[1]Received June 8, 1981; accepted July 10, 1981.

[2]Supported by Public Health Service grants CA-25190 and CA-17970 (J. H. Fetting)(National Cancer Institute) and HL-19248 and HL-00280 (National Heart, Lung, and Blood Institute) from the National Institutes of Health, Department of Health and Human Services, and IM-11Q from the American Cancer Society. Dr. Fetting is the recipient of a Regular American Cancer Society Fellowship.

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[4]The inductive plethysmograph (Respitrace) used in this study was graciously lent to us by Mr. William Gruen, Ambulatory Monitoring, Inc.

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Recordings of thoracic and abdominal wall movements during vomiting were always accompanied by those of thoracic venous pressure, which served as our standard criterion of emetic activity (6,7). Thoracic venous pressure was accessible through a jugular catheter, which was temporarily placed in the decerebrate animals at the time of surgery and in the intact animal some days earlier, under general anesthesia. In the intact animal, the catheter was exteriorized through a stab wound on the dorsum of the neck. All recordings were made through appropriate transducers and preamplifiers on a Gould model 2400, four-channel oscillograph and on a Hewlett Packard model 3690 FM instrumentation tape recorder. Recording gains were adjusted so that normal ventilatory excursions encompassed $\leq 5\%$ of the full channel width. A videotape recording was also made of the intact cat for later stop-frame analysis. A split-screen attachment allowed simultaneous viewing of the oscillographic traces and body wall movements.

Positioning of the animals during emesis varied. One decerebrate animal was fixated in a head holder that allowed opening of the mouth. The animal was permitted to rest on its abdomen while vomiting. The remaining three decerebrate cats were suspended by their heads and pelvic girdles from a horizontal bar. This permitted normal flexion of the animals during vomiting and avoided dimensional restriction of the body walls. The intact cat was unrestrained and postured normally.

Emesis was induced in the decerebrate animals with alkavervir (Veriloid) (25–50 μg/kg im) and in the intact animal with deslanoside (160 μg/kg iv).

The purpose of this study was to determine whether some characteristic of body wall dynamics might provide an objective end point for discerning emetic activity. Accordingly, we compared two methods of monitoring body wall movements for their suitability in this application.

### One-Dimensional Magnetometry

This technique consists of applying pairs of tuned coils to opposite sides of the torso. The recorded output of the dual-channel device used in this study consisted of a DC voltage that was proportional to the distance between each pair of coils. For the purpose of this study, no significance was attached to the nonlinear characteristics of the instrument. The timing and the relative direction of movement of the coils with respect to each other were of primary interest, and no attempt was made to quantify volumetric changes in the visceral compartments. Rather, the relative magnitude of emetic efforts vis-a-vis those seen during resting breathing were considered in evaluating the usefulness of the method.

Magnetometer coils were positioned at four general locations to monitor body wall movement. The units were placed opposite each other on the lateral aspects of the chest and were centered approximately at ribs 3 and 4 or at 9 and 10. Abdominal dimensions were measured either horizontally across the lower abdominal or midabdominal region or dorsoventrally by fixing one coil on the back, with the mating coil placed directly below it on the rectus muscle. Dimensions could be monitored at only two locations at one time. However, there was generally sufficient time between emetic episodes to allow repositioning of the coils so that readings from various positions could be compared in the same animal.

### Inductive Plethysmography

Two infant-size coils from an inductive plethysmograph (Respitrace, Ambulatory Monitoring, Inc, Ardsley, NY) were placed around the upper thoracic and midabdominal regions of two cats. This instrument produces voltage outputs proportional to the enclosed cross-sectional areas of each coil and has been shown to be a reliable monitor of thoracic and abdominal volume changes in man (8). No volume calibration was performed in the present investigation since only qualitative assessment of rib cage and abdominal volume changes with retching and expulsion was required.

## RESULTS

### Body Wall Movements During Emesis

Thirty-six emetic episodes were monitored in the five experimental animals of this series. In every case the thoracic venous pressure trace served not only to document the occurrence of vomiting but to distinguish between retching and expulsive body wall movements. Thoracic venous pressure changes and chest and abdominal movements as measured by the inductive plethysmograph are compared during a single emetic episode in figure 1. This single response, which occurred over a 30-second period, characterizes the relationships of central venous thoracic pressure and body wall movement during vomiting. In this and all subsequent recordings of emesis, amplifier gains of all channels were reduced to suppress normal resting ventilatory excursions. As seen in this figure, thoracic venous pressure typically distinguished between the 21 retching efforts (negative pulses) and the single terminal expulsive maneuver (positive pulse). Both the chest and abdomen displayed characteristic movements of large

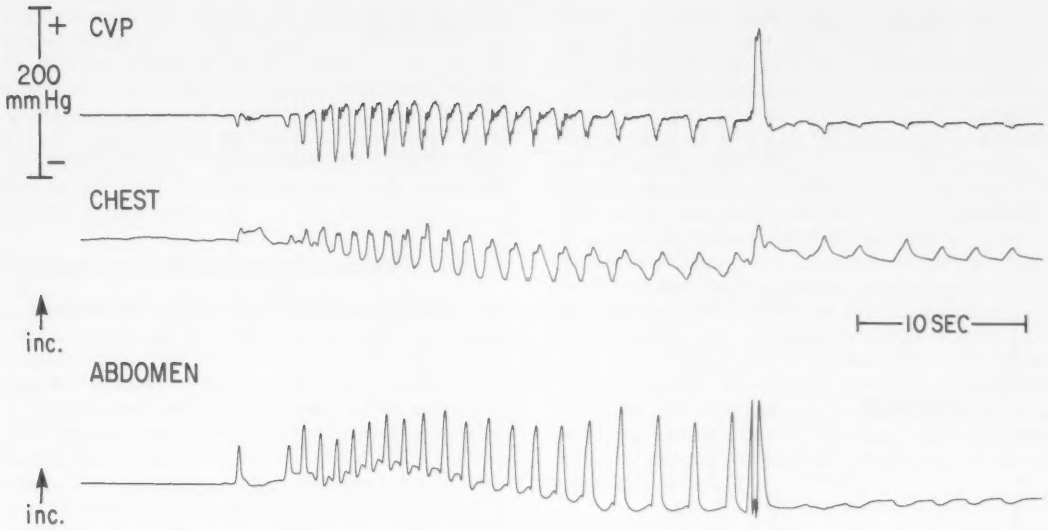


FIGURE 1.—Oscillographic recording of a single emetic episode in a decerebrate cat. Thoracic venous pressure trace characteristic of emesis is compared with chest and abdominal body wall movements. Trace of central venous pressure (CVP), measured by way of a jugular catheter, shows repeated negative spikes typical of retching and single terminal pulse, denoting expulsion. Resting ventilatory excursions of chest and abdomen were suppressed to ≤ 5% of full channel width. Body wall movements were recorded by the inductive plethysmograph technique. Increases in dimensions of chest and abdomen are indicated by an upward deflection. Hyperpnea is evident following the emetic episode.

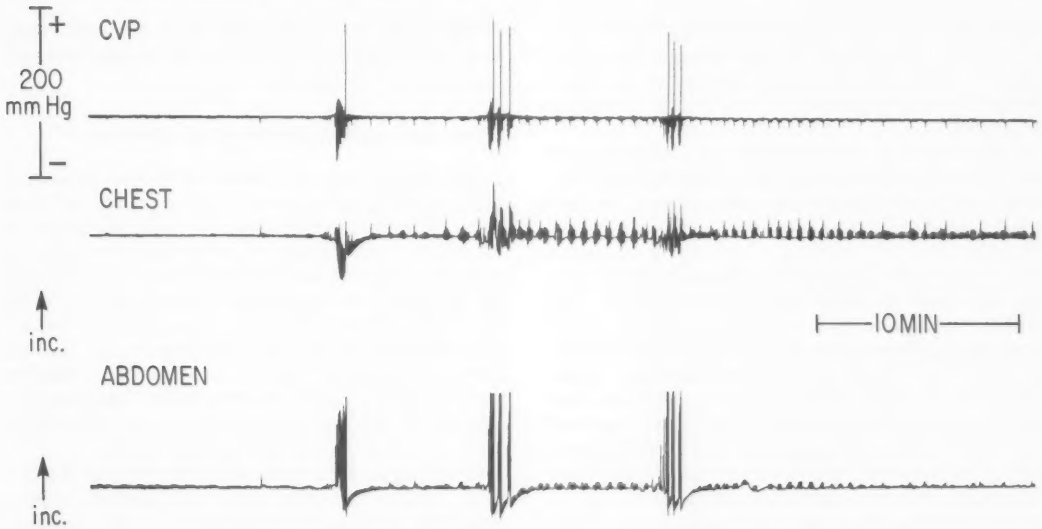


FIGURE 2.—Continuous 45-min oscillographic trace showing 7 emetic episodes in a decerebrate cat. Inductive plethysmographic recording of abdominal movement indicates large increases in abdominal dimensions coinciding with standard vomiting signal as recorded in the central venous (thoracic) pressure (CVP) trace. Movements of chest wall during emesis are partially obscured by an augmented and periodic ventilatory pattern produced by the emetic agent, Veriloid. Resting ventilatory movements are suppressed into baseline prior to occurrence of first emetic episode. Increase in torso dimension is represented by an upward pen deflection.

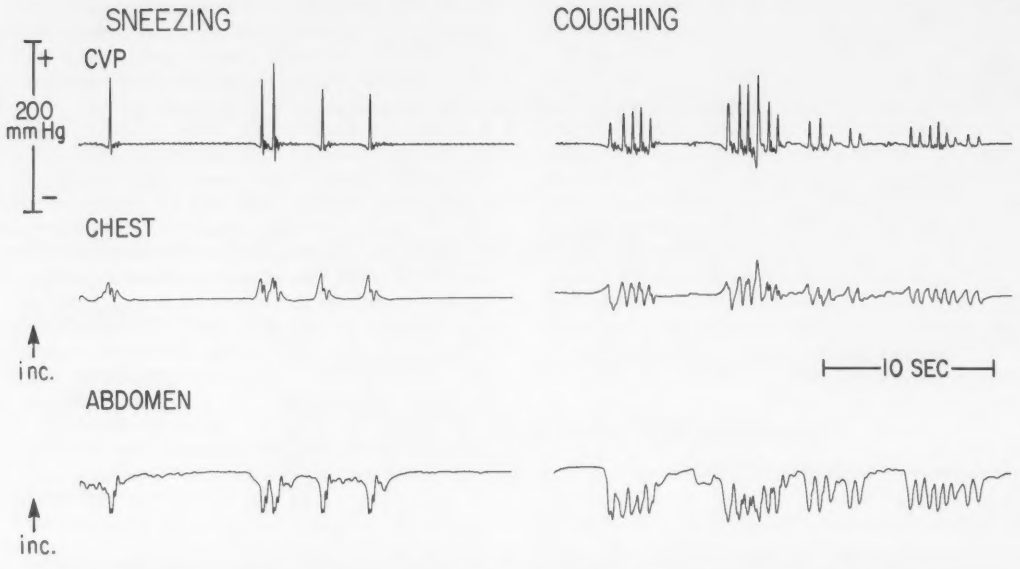


FIGURE 3.—Sneezing and coughing activity as monitored in a decerebrate animal by the magnetometer technique. Movements of chest and abdomen were recorded at gains of 2½ and 5 times greater, respectively, during these maneuvers than those used for recording emesis. In contrast to what was seen during vomiting, the abdomen showed a reduction in size during these activities, underscoring the expiratory nature of these events. Conventions are as in fig 1.

magnitude, which were synchronized with the venous pulses. In contrast to the pressure measurement, however, there was no distinction in either the direction or magnitude of retching or expulsion movements.

Tonic as well as cyclic changes in abdominal and thoracic wall positions were noted during vomiting, but it was primarily the cyclic movements, as seen in the abdominal trace, that most clearly denoted the occurrence of vomiting. Contrary to expectation, the abdomen displayed a dramatic increase in size with each retching and expulsive effort. The magnitude and direction of these movements, compared with those observed during normal ventilation, provided a clear index of emetic activity. These abdominal tracings were sufficiently distinct that they remained clearly evident even when recorded at low paper speed. Figure 2 is a 45-minute segment of a record in which the central venous pressure and abdominal movements signify the occurrence of seven emetic episodes. Even at this paper speed, which is suitable for long-term monitoring, the abdominal excursions are sufficiently unique to serve as objective end points of emetic activity. Movements of the chest, on the other hand, were found to be unsuitable for this purpose. Although they were of a consistent and predictable direction, they were of smaller magnitude, started from a more labile base, and were more easily obscured by augmented ventilatory activity (fig 2).

## Other Spasmodic Respiratory Activity

Abdominal wall movements of the cat associated with retching and expulsion differ sufficiently from those of normal ventilation to serve as indices of emetic activity. However, other episodic behavior such as coughing and sneezing might be mistaken for vomiting. We recorded these events in one decerebrate animal, using the magnetometer method and compared them with vomiting (fig 3). This animal had exhibited long-standing sneezing behavior, which persisted after decerebration. Coughing was induced by placing approximately 1 ml of tap water from a syringe at the back of the throat.

Coughing and sneezing were detected in the thoracic venous pressure trace as positive spikes. Sneezing appeared as a singular event, whereas coughing was seen as bursts of spasmodic activity. Thoracic and abdominal body wall movements differed from those seen during emesis in two major respects. First, the magnitude of the excursions of both traces, although greater than those seen during resting ventilation, was considerably less than

that observed during emesis. (The amplifier gains of the thoracic and abdominal recordings shown in fig 3 were increased by 2½ and 5 times, respectively, to show detail.) Second, abdominal traces during coughing and sneezing clearly showed a reduction in dimension that was commensurate with expiratory effort. The size and direction of these movements are in clear contrast with those observed during vomiting.

### Comparison of Monitoring Techniques

Emesis, as judged by recordings of body wall movement, could be detected by both the one-dimensional magnetometric and by the plethysmographic methods. Both monitoring techniques revealed characteristic movements, which correlated with thoracic pressures recorded from central veins. Additionally, the observation that the abdomen increased in dimension during retching and expulsion was verified by both techniques. Magnetometer recordings of abdominal movements, however, lacked the magnitude that characterized plethysmographic traces and that made vomiting efforts so distinct from background (nonemetic) activity (fig 4). Magnetometer tracings that were comparable in magnitude to those obtained by the plethysmographic method were seen in only one animal. This cat was allowed to vomit while lying on its abdomen. The dorsoventral constraint thus imposed allowed greater lateral displacement in the plane of the sensing coils.

The one-dimensional monitoring device was subject to artifact and the type of interpretational errors illustrated in figure 4. Vocalization was observed at the point where the slow positive wave is evident in the thoracic venous pressure trace. The abdominal recording indicates a dimensional increase during the execution of this forced expiratory maneuver. Subsequent stop-frame videotape analysis of this and similar events clearly revealed that the sensing coils, placed on the lateral aspects of the abdomen, moved outward during vocalization, while the ventral aspect of the body wall moved briskly inward. In contrast, tracings obtained by inductive plethysmography were remarkably stable; they exhibited excellent signal differentiation between normal and emetic activity and were apparently artifact-free.

## DISCUSSION

The importance of the abdominal muscles in vomiting has long been appreciated (9,10), but the coordinated forces that effectuate the response have only recently been documented (4,5).

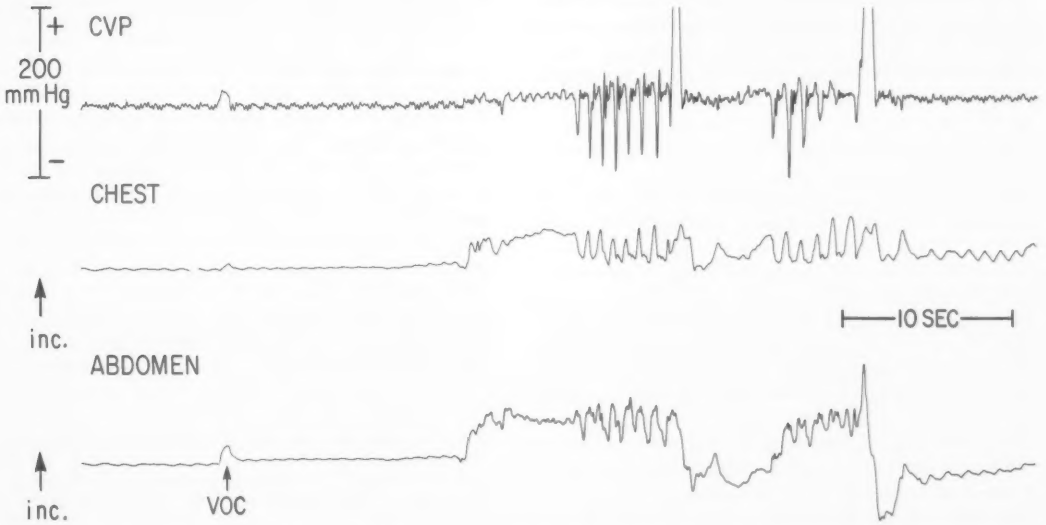


FIGURE 4.—Tracings of 2 emetic episodes recorded from an unanesthetized intact cat by the magnetometer technique. In this instance, abdominal sensors were placed on the animal's flanks midway down the abdomen. Body wall movements, although recognizable as measured by this one-dimensional method, are less clearly defined than those recorded by the inductive plethysmograph. Potentially misleading traces were seen with this recording technique, such as the increase in abdominal dimension during vocalization (VOC). Emesis was induced with deslanoside, 160 μg/kg iv. Conventions are as in fig 1.

Concurrent negative thoracic and positive abdominal pressures generate transdiaphragmatic pressure differentials of up to 200 mm Hg during retching. In expulsion, thoracic pressure shifts from negative to positive, and the abdominal pulse becomes greater and more sustained. It is not surprising that abdominal wall movements reflect emetic activity; it is only the direction of those movements that remains unexplained. This is especially true since electromyographic studies in the cat (4) and the dog (11) have shown the diaphragm and the abdominal muscles to be simultaneously active during both retching and expulsion. What has not been clear is the relative involvement of these muscle groups.

The increase in abdominal dimension recorded during retching and expulsion emphasizes the exquisite control exercised during these processes. During retching the diaphragm descends, working against a closed glottis, developing the negative thoracic pressures. The present study reveals that this diaphragmatic effort is exerted against tonic, but nonetheless relenting, abdominal muscle contractions. This kind of highly tuned coordination between the diaphraghm and the abdominal muscles is required to explain the simultaneity of the three observed phenomena: rarefaction of the thoracic contents, increased abdominal pressure, and increase in abdominal volume.

If these emetic processes hold across species lines the present study in the cat indicates that monitoring for characteristic body wall movements, especially those of the abdomen, might serve as a useful and noninvasive method for quantifying vomiting in man.

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# Acute Vascular Episodes Associated With Therapy for Carcinomas of the Upper Aerodigestive Tract With Bleomycin, Vincristine, and Cisplatin [1]

Brief Reports

Lawrence J. Kukla,[2,*] William P. McGuire,[2] Thomas Lad,[2] and Manuel Saltiel[3]

## SUMMARY

**Twenty-nine patients were treated for carcinoma of the head and neck with a combination of cisplatin, vincristine, and bleomycin. Four of these patients had acute vascular episodes (three had cerebrovascular accidents and one developed Raynaud's phenomenon) during or after treatment. Two additional patients with a similar history and course are also included for analysis. Three of these six patients had known underlying cardiovascular disease, which may have been a contributing risk factor. A reinstitution of bleomycin infusion produced a recurrence of the Raynaud's phenomenon in the patient who had developed that toxic effect.**

[Cancer Treat Rep 66:369–370, 1982]

At our institution, we have treated patients with advanced head and neck cancer, either before local therapy or after recurrence, with a combination of cisplatin, vincristine (VCR), and bleomycin (BLM) [a regimen similar to that used by Elias et al (1)]. The regimen we used consisted of: (*a*) 100 mg/m² of cisplatin as a 24-hour infusion on Day 1; (*b*) 1 mg/m² of VCR as an iv bolus on Day 3; and (*c*) 10 units/m² of BLM as an iv bolus on Day 3, then 10 units/m² as an iv infusion over 24 hours on Days 3–6. Twenty-nine patients were treated with this regimen (a total of 35 treatments given) from July 1979 to December 1980. Table 1 lists four patients who had acute vascular episodes during or after this treatment. Other patients included in this report, treated without VCR, had a similar course. Table 2 lists the observed vascular problems, all of which occurred during the first treatment course with this regimen.

The rate of occurrence of cerebrovascular accidents (CVAs) in these patients with head and neck cancer (three of 29) is alarming in light of the close temporal relationship between their occurrence and the administration of chemotherapy. Previously published studies employing similar regimens have not reported this form of drug toxicity. However, cerebral and/or vascular toxic effects have been reported with the use of these drugs. VCR neurotoxicity is probably the most widely known of all of these associations. Cisplatin has also been reported to produce neurologic side effects which might represent direct neurotoxicity (2). Isolated vascular lesions have been associated with VCR [acute myocardial infarctions (3–5)] as well as with BLM [Raynaud's phenomenon (6–8) and thrombosis (9)]. Vascular lesions also appear to be an early pathologic finding in BLM-induced pulmonary fibrosis (10). These observations, however, fail to explain the mechanism of injury in our reported cases.

The exact agent (or agents) responsible for the observed effects is also not apparent. Due to the nature and severity of the vascular events, we were able to observe significant improvement in only *one* patient (No. 3); therapy was subsequently reinstituted. In this patient, the BLM infusion was resumed, and the vascular event (Raynaud's phenomenon) recurred after 6 hours of therapy. After the second discontinuation of treatment, the digital lesions again began to resolve. Although this type of "rechallenge" was not performed for patients with *cerebral* vascular events, it does suggest the strong temporal relationship of the effects to the BLM infusion, which we observed in all of the patients. This does not exclude, however, the possibility that the

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TABLE 1.—Patient data

| Patient No. | Race/sex | Age (yrs) | History of cardiovascular disease | Cancer site |
|---|---|---|---|---|
| 1 | B/M | 61 | Aortic insufficiency | Larynx |
| 2 | W/M | 54 | Aortic insufficiency | Oropharynx |
| 3 | W/M | 64 | None known | Pharynx |
| 4 | W/M | 55 | None known | Tonsil |
| 5* | B/M | 58 | None known | Esophagus |
| 6* | B/M | 60 | Prior left CVA | Esophagus |

*Did not receive VCR.

TABLE 2.—Acute vascular events

| Patient No. | Acute event | Onset of symptoms | Survival |
|---|---|---|---|
| 1 | Left CVA | After 8 hrs of BLM | 4 days after CVA |
| 2 | Frontal lobe infarction | After 12 hrs of BLM | 8 days after CVA |
| 3 | Raynaud's phenomenon | After 14 hrs of BLM | 13 days (aspiration) |
| 4 | Right CVA | 12 hrs after completion of treatment | 16 days after CVA |
| 5 | Right anterior parietal infarction | After 48 hrs of BLM | 30 days after CVA |
| 6 | Right 7th cranial nerve palsy; aphasia | 64 hrs after completion of treatment | Alive |

toxicity is multifactorial and an effect of a synergistic drug interaction.

The existence of underlying cardiovascular disease may be equally important in the pathogenesis of these events: two of six patients were known to have underlying valvular heart disease. Transient hypertension (reported with cisplatin), hypotension (from vomiting or fever), vasospasm, or thrombosis could all theoretically exacerbate subclinical perfusion defects. We did not observe hypertensive episodes in these patients, nor did they become volume-contracted during treatment. However, the other possibilities have not been investigated. Additional studies are needed to determine their roles in the pathophysiology of this unusual association between combination chemotherapy and acute vascular episodes.

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# Clinical Trial of Chlorozotocin, DTIC, and Dactinomycin in Metastatic Malignant Melanoma[1,2]

Brief Reports

Michael K. Samson,[3,*] Laurence H. Baker,[3] Glenn Cummings,[3] Robert W. Talley,[4] Bruce McDonald,[3] and Dinyar B. Bhathena[3]

## SUMMARY

**Thirty evaluable patients with metastatic malignant melanoma were treated with a combination of chlorozotocin, DTIC, and dactinomycin. One complete and four partial responses were achieved, for an overall response rate of 17%. Cumulative myelotoxicity was noted, as well as universal gastrointestinal intolerance. One patient developed probable drug-related nephrotoxicity. A survival advantage was noted for responding patients. We conclude that the addition of chlorozotocin did not improve response rate or response duration and that further trials with this drug combination cannot be recommended.**

[Cancer Treat Rep 66:371–373, 1982]

Chlorozotocin, a chloroethyl nitrosourea, was selected for clinical trial on the basis of its curative antitumor activity in the L1210 leukemia system and of its ability to produce twice the alkylation of BCNU in equimolar concentrations at doses that produce little or no bone marrow suppression (1–4). Clinical trials with this agent in patients with malignant melanoma demonstrated objective clinical remissions in visceral, subcutaneous, and lymph node metastatic sites, with minimal myelosuppression (5,6).

An extensive analysis of single-agent effectiveness in the treatment of malignant melanoma showed that DTIC, with an overall response rate of 23% (7), remains the single most useful agent in this disease. Duration of response is 6 months, with survival approaching 1 year. Attempts to improve response rate, response duration, and survival by using combination drug regimens containing DTIC have failed to conclusively show an advantage over DTIC alone (7). We have previously reported our results of a clinical trial using intermittent bolus administration of DTIC and dactinomycin (8): all our responses (five patients, 23%) occurred in patients with predominantly visceral disease and at nonmyelosuppressive doses. In view of the clinical activity of chlorozotocin in melanoma, at doses that were minimally myelotoxic, it appeared reasonable to combine it with DTIC and dactinomycin in untreated patients with metastatic malignant melanoma.

## MATERIALS AND METHODS

All patients who entered this study had histologic documentation of malignant melanoma with evidence of dissemination. No patient had received prior systemic chemotherapy. All were informed as to the investigational nature of the study and gave written consent in accordance with our institutional policy. Additional eligibility criteria included the presence of measurable disease; expected survival of at least 6 weeks; adequate renal and hepatic functions defined as BUN $\leq$ 25 mg/100 ml, creatinine $\leq$ 1.5 mg/100 ml, and bilirubin $\leq$ 2.5 mg/100 ml; wbc count $\geq$ 4000/mm$^3$; and platelet count $\geq$ 100,000/mm$^3$.

While receiving therapy, patients were evaluated with serial wbc and platelet counts, SMA-17 chemistry panel, chest roentgenograms, tumor measurements, and performance status determination.

DTIC was administered iv over 15–20 minutes at a dose of 750 mg/m$^2$, followed by bolus iv dactinomycin at a dose of 1.25 mg/m$^2$ and chlorozotocin at a dose of 100 mg/m$^2$. Chlorozotocin was repeated at 6-week intervals, while DTIC and dactinomycin were given every 21 days. Poor-risk patients (prior radiation therapy to major bone marrow-bearing areas or $\geq$ 65 years old) received DTIC at a dose of 600

[1]Received Dec 29, 1980; revised June 15, 1981; accepted June 24, 1981.

[2]Supported by contract N01-CM-67105 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and Harper-Grace Hospitals, Detroit, MI.

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mg/m², dactinomycin at a dose of 1 mg/m², and chlorozotocin at a dose of 90 mg/m².

Criteria of response were standard. A complete remission was defined as the disappearance of all clinical evidence of tumor for a minimum of 4 weeks, together with the disappearance of all tumor-related symptoms. A partial remission was defined as a $\geq$ 50% decrease in the sum of the products of the largest perpendicular diameters lasting at least 4 weeks in the absence of a significant increase in any one lesion or the appearance of new lesions. Progressive disease was defined as an unequivocal increase (> 25%) in any measured lesion or the appearance of new lesions. Patients were considered to have an early death if death from tumor-related causes occurred within the first 2 weeks of therapy.

### Patients

Patient characteristics are summarized in table 1. A total of 36 patients were entered in this study, with 30 patients evaluable for response. Three patients had early deaths, and three were lost to followup. Metastatic sites are presented in table 1.

TABLE 1.—Patient characteristics

| Characteristic | Risk | |
|---|---|---|
| | Good | Poor |
| No. of patients entered/ evaluable for response | 26/21 | 10/9 |
| No. of males/females | 13/13 | 5/5 |
| Median age in yrs (range) | 45 (18–63) | 68 (31–80) |
| Median Karnofsky performance status (range) | 90 (55–90) | 80 (55–90) |
| No. of patients with— | | |
| Primary sites | | |
| Eye | 0 | 2 |
| Extremities | 9 | 4 |
| Head and neck | 5 | 1 |
| Trunk | 10 | 2 |
| Vulva | 0 | 1 |
| Unknown | 2 | 0 |
| Metastatic sites* | | |
| Skin | 13 | 4 |
| Lymph nodes | 13 | 3 |
| Lung | 13 | 1 |
| CNS | 6 | 0 |
| Bone | 4 | 1 |
| Liver | 3 | 4 |

*Most patients had multiple metastatic sites.

## RESULTS

### Response and Survival

Of the 30 patients evaluable for response, one had a complete response and four had a partial response (all treated at good-risk doses), for an overall response rate of 17%. Median duration of response was 31 weeks (range, 16–39). Responses were achieved in patients with lung, pelvic, liver, nodal, and subcutaneous metastases. For responding patients, median survival was 84+ weeks, compared to 24 weeks for nonresponding patients. This difference was statistically significant ($P < 0.05$). Median survival for the entire group of patients was 24 weeks.

### Toxicity

The major toxic effect was myelosuppression, with a median wbc count nadir of 2.6 × $10^3$/mm³ (range, 0.3–5.4) and a median platelet count nadir of 87 × $10^3$/mm³ (range, 1–306) in good-risk patients; for poor-risk patients, median wbc count nadir was 3.2 × $10^3$/mm³ (range, 1.0–8.2), and platelet count nadir was 139 × $10^3$/mm³ (range, 18–348). Overall, 29% of the patients developed grade 3 (< 2 × $10^3$/mm³) or greater leukopenia and 32% developed grade 3 ($\leq$ 50 × $10^3$/mm³) or greater thrombocytopenia. For those patients who manifested myelosuppression, there was a trend toward cumulative toxic effects with each succeeding 6-week cycle of therapy.

Major nonhematologic toxicity consisted of moderate to severe nausea and vomiting in 80% of the patients, accompanied by diarrhea in at least 26%. Two patients manifested maculopapular skin eruptions, which responded to antihistamines and short-term administration of corticosteroids.

Following a total cumulative chlorozotocin dose of 390 mg, a 43-year-old female patient had an acute hypertensive episode accompanied by generalized edema, proteinuria of 6 g/24 hours, elevated serum creatinine of 3.1 mg/100 ml, and microscopic hematuria. Evaluation showed no infectious or autoimmune etiologies. A renal biopsy was performed with the patient's consent. Histologic and electron microscopic studies showed that glomerular necrosis, hypercellularity, and interstitial inflammation were absent; immunofluorescence studies were negative. Tubular changes were noted and characterized by moderate atrophy, occasional dilatation, anisonucleosis, hyperchromatism, and prominent nucleoli; these findings were not incompatible with effects noted secondary to chemotherapeutic agents. Ultrastructure studies demonstrated an accumula-

tion of predominantly electron-lucent material, split loops, and mesangial interposition.

## DISCUSSION

Chlorozotocin, a chloroethyl nitrosourea rationally synthesized and subsequently shown to have superior alkylating activity to other nitrosoureas at nonmyelosuppressive doses, had shown clinical activity against malignant melanoma. Because of these important in vivo and in vitro observations, we proceeded to combine chlorozotocin with DTIC and dactinomycin. Our results indicate lack of a significant advantage in terms of response rate (17%; 95% confidence limits, 6%–35%) or response duration for the three-drug combination, compared to our prior treatment regimen that did not contain chlorozotocin. There was, however, a modest 3-week improvement in overall survival of our patients, compared to overall survival with DTIC and dactinomycin (25 vs 22 weeks, respectively). In addition, we observed cumulative myelosuppression with the three-drug combination, and one patient had possible drug-induced nephrotoxicity.

Based on our observations in this trial, we cannot recommend further clinical studies with chlorozotocin combined with DTIC and dactinomycin in patients with disseminated malignant melanoma.

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Brief Reports

# High-Dose Cisplatin for Lung Cancer [1]

**Vinod K. Bhuchar[2,*] and Victor J. Lanzotti[2,3]**

Oncologists continue to employ cisplatin in chemotherapy programs for lung cancer despite the fact that there are no convincing data supporting its value in the treatment of this disease. Published data show that single-agent cisplatin in conventional dose (50–75 mg/m²) for lung cancer is associated with a response rate of 5% (1). The objective of the present study is to determine if high-dose cisplatin with parenteral fluids and mannitol-induced diuresis (2) is associated with a more substantial rate of response.

## MATERIALS AND METHODS

Thirty-three selected patients with lung cancer (adenocarcinoma, 16; squamous cell, five; large cell, three; and small cell, nine) were treated with high-dose cisplatin and parenteral fluids plus mannitol-induced diuresis. All patients selected for study had at least one measurable or evaluable lesion that had not been included in a prior radiotherapy field, favorable Zubrod symptom status (0–2), creatinine clearance ≥ 60 ml/minute, normal audiogram in spoken-voice range (500–2000 Hz), and anticipated survival > 8 weeks (3). There were 26 patients with extensive disease and seven patients with limited disease. Ten patients had received prior chemotherapy, ten had received prior chemotherapy and radiotherapy, and four had received prior radiotherapy alone. All of the nine patients with small cell lung cancer had received extensive prior chemotherapy. Nine of the 24 patients with non-small cell lung cancer had received no previous treatment, and four had received radiotherapy alone. Thus, 13 patients with non-small cell lung cancer had received no prior chemotherapy.

Cisplatin dosage was 100, 120, or 140 mg/m² in 200 ml of normal saline over 2 hours every 28 days. The 33 study patients received 61 cycles of chemotherapy (30 cycles at a dose of 100 mg/m², 19 cycles at 120 mg/m², and 12 cycles at 140 mg/m²). Patients were given parenteral fluids, mannitol, and cisplatin as outpatients or inpatients, depending on bed availability at the time of the first course of chemotherapy. Inpatients received 2 liters of prehydration fluid beginning 12 hours before cisplatin and 5 liters of parenteral fluid with 40 g of mannitol/liter over the 24 hours following initiation of chemotherapy. Outpatients received 0.5 liters of prehydration fluid beginning 2 hours before cisplatin and 1 liter of parenteral fluid with 40 g of mannitol over the 4 hours following chemotherapy. In addition, 12.5 g of mannitol was given as an iv bolus to all patients just prior to administration of cisplatin. Nineteen patients received 34 cycles of chemotherapy according to the 36-hour schedule, while 14 patients received 27 cycles of chemotherapy according to the 6-hour schedule. Blood cell counts and serum creatinine were determined at weekly intervals. Audiograms and measurement of creatinine clearance were performed prior to each course of chemotherapy.

Complete response was defined as the disappearance of all measurable signs and symptoms of disease. Partial response was defined as a ≥ 50% reduction in the product of two perpendicular diameters or substantial improvement of evaluable lesions (4).

## RESULTS

One patient showed complete response to chemotherapy with high-dose cisplatin. This patient had recurrent small cell lung cancer that was manifested by a large left-pleural effusion, which completely resolved after two courses of cisplatin at a dose of 140 mg/m². There were no partial responses. Thus, one of nine patients with small cell lung cancer and none of 24 patients with non-small cell lung cancer responded to high-dose cisplatin chemotherapy.

Nausea, vomiting, and persistent anorexia were severe. Serum creatinine elevation > 1.5 mg/100 ml was observed in four of 29 (13%) chemotherapy cycles at a dose of 100 mg/m², in four of 16 (25%) at 120 mg/m², and in five of ten (50%) at 140 mg/m². Serum

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creatinine > 2.5 mg/100 ml was observed with one cycle of chemotherapy at a dose of 100 mg/m$^2$ and with three cycles of chemotherapy at a dose of 140 mg/m$^2$. Twenty-nine patients were evaluable for ototoxicity, defined as a decline of at least 10 db in both ears or 15 db in one ear. Hearing loss at 4000–8000 Hz occurred in 14 patients; four of these patients also showed hearing loss at 2000 Hz. A decrease in hemoglobin of at least 2.0 g/100 ml was observed following seven cycles of chemotherapy. Total wbc count nadirs < 3500/mm$^3$ and a platelet count nadir < 100,000/mm$^3$ followed four cycles and one cycle of chemotherapy, respectively. There were no detectable differences in toxic effects between the 36- and 6-hour schedules.

## DISCUSSION

Rossof et al (1) reviewed the literature and their own experience and found only two responses among 39 patients with lung cancer who received cisplatin in conventional dosage. The present study confirms that cisplatin is relatively ineffective as a single agent for patients with lung cancer, even when it is employed in maximally tolerated dosage. The occurrence of only one response among 33 patients with lung cancer and no response among 24 patients with non-small cell lung cancer is particularly significant in view of the fact that study patients were selected on the basis of favorable symptom status, evaluability for response, and minimal prior therapy. The argument that cisplatin is ineffective for small cell lung cancer is somewhat less convincing because one complete response did occur among the nine previously treated patients with small cell lung cancer in this study. However, the available data convincingly indicate that the response rate of non-small cell lung cancer to single-agent cisplatin is extremely low, even when high doses of cisplatin are used in favorably selected patients.

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# Phase II Study of a High-Dose Regimen of Cyclophosphamide and Prednisolone in Advanced Non-Hodgkin's Lymphoma of Favorable Histologic Type[1]

Brief Reports

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## SUMMARY

**Fifty-seven courses of cyclophosphamide (2.5–5.0 g/m²) and prednisolone (1.0 g/m² × 5) were given to 22 patients with advanced stage IV non-Hodgkin's lymphoma of favorable histology. Six patients (27%) had a complete response (CR) (median duration, 10.1 months), and six (27%) had a partial response (median duration, 3.0 months). All patients in whom CR was achieved had a previous disease pattern of remission and relapse, and no patient refractory to previous therapy had a CR. This association of CR to disease pattern was statistically significant ($P < 0.0001$). There was no difference in the survival of complete and partial responders. There was a trend to a higher response rate and statistically significant survival advantage ($P < 0.05$) for patients with the nodular histologic types. The transient nature of response and the toxicity of the regimen, with four therapy-related deaths, render it unsuitable as routine therapy.**

[Cancer Treat Rep 66:377–380, 1982]

Patients with non-Hodgkin's lymphomas of favorable histologic types (1) have a median survival of 5–7 years; patients with the nodular subtypes have a better prognosis (2,3). There has been little survival advantage demonstrated from combination chemotherapy in comparison to single agents, although more complete remissions are obtained with combination therapy (1,4). However, the responses are not durable (4,5), in contrast to those achieved in unfavorable histologic subtypes (6). In a previous study (1) the median duration of complete remission was identical to that of good partial remission, suggesting that patients assessed as being in complete remission were not necessarily disease-free. This led us to investigate the role of intensive cyclophosphamide (CTX) and prednisolone (Pred) therapy in selected patients with advanced disease.

## PATIENTS AND METHODS

Twenty-two patients (11 males and 11 females; median age, 50 years) were selected for investigation between November 1977 and August 1979. Full details are shown in table 1. The patients were stratified into three clinical groups. Group 1 consisted of seven previously untreated patients with bulky disease (stage II or IV with confluent nodal masses > 2.5 inches in diameter at multiple sites) at first presentation. Group 2 contained nine patients refractory to previous chemotherapy with CTX, vincristine, and Pred (CVP) or chlorambucil (CB) administered as previously described (1). Two patients had also received radiotherapy. Group 3 consisted of six patients who had achieved complete remission during prior treatment with CVP or CB. All patients had advanced disease at second or third relapse on entry to the study. The histologic diagnosis was reviewed and repeat biopsies were performed before therapy in all previously treated patients.

The patients were premedicated with allopurinol, and hydration was ensured by an iv infusion of saline over the first 24–48 hours. On Day 1, CTX (2.5 g/m² iv) was given with methylprednisolone (1 g/m² iv/day) from Days 1 to 5. Treatment was repeated at about 21–28-day intervals for three cycles and, where possible, responders received a total of six cycles. The CTX dose was escalated in subsequent courses in increments of 1.25 g/m² to a maximum dose of 5 g/m², toxicity permitting.

[1]Received June 5, 1980; revised July 16, 1981; accepted Aug 6, 1981.

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TABLE 1.—Details of phase

| Group | Disease description | Patient No. | Age (yrs) | Sex | Histology* | | Disease stage | Bone marrow |
|---|---|---|---|---|---|---|---|---|
| | | | | | Rappaport | Kiel | | |
| 1 | Bulky disease; no prior therapy | 1 | 63 | F | DWDL | lcd | IV | + + + |
| | | 2 | 33 | F | NPDL | cbccf | III | |
| | | 3 | 55 | M | NPDL | cbccf | IV | + + + |
| | | 4 | 60 | M | NPDL | cbccf | IV | + + |
| | | 5 | 62 | F | DPDL(i) | T zone | III | |
| | | 6 | 44 | M | NPDL | cbccf | IV | + + |
| | | 7 | 58 | F | NPDL | cbccf | IV | + |
| | | | 53.6‡ | | | | | |
| 2 | Refractory disease | 8 | 32 | M | DPDL(i) | lpcd | IV | |
| | | 9 | 61 | M | DWDL | lcd | IV | + + |
| | | 10 | 55 | M | DWDL | lcd | IV | + + + |
| | | 11 | 38 | F | NPDL | cbccf | IV | + |
| | | 12 | 68 | M | DPDL(i) | cbccd | IV | + + + |
| | | 13 | 57 | M | NPDL | cbccf | IV | |
| | | 14 | 55 | M | DWDL | lcd | IV | + + + |
| | | 15 | 32 | F | NPDL | cbccf | IV | + + |
| | | 16 | 46 | F | DPDL(i) | cbccd | IV | + + + |
| | | | 49.3‡ | | | | | |
| 3 | Remitting and relapsing disease | 17 | 58 | F | NPDL | cbccf | IV | |
| | | 18 | 48 | F | NPDL | cbccf | IV | + + |
| | | 19 | 31 | M | NPDL | cbccf | IV | + + + |
| | | 20 | 45 | F | NPDL | cbccf | IV | + |
| | | 21 | 34 | F | NPDL | cbccf | IV | + + |
| | | 22 | 34 | M | NPDL | cbccf | IV | + |
| | | | 41.6‡ | | | | | |

*DWDL = diffuse well-differentiated lymphocytic, NPDL = nodular poorly differentiated lymphocytic, DPDL(i) = diffuse poorly differentiated lymphocytic intermediate, lcd = lymphocytic diffuse, lpcd = lymphoplasmacytoid diffuse, cbccf = centrocytic centroblastic follicular, cbccd = centrocytic centroblastic diffuse, T zone = T zone lymphoma.

†D = death during therapy, NR = no response.

‡Mean value.

§Median value.

¶OPAL = vincristine, prednisolone, doxorubicin, and L-asparaginase.

Complete response (CR) was defined as the disappearance of all assessable disease, and investigation included repeat bone marrow biopsy. Partial response (PR) was defined as a > 50% reduction in the sum of the products of the largest diameter and its perpendicular for all measurable lesions, with no appearance of any new lesions.

## RESULTS

Twenty-two patients received 57 courses of therapy (range, one to six/patient; mean, 2.6/patient). Transient and often dramatic reduction in disease was seen in 19 of the patients at Day 7–14 after therapy; however, by Day 21–28 the disease had frequently returned. CR was achieved in six patients (27%); they were cleared of clinical disease with one course of treatment (four patients) or two courses (two patients). The formal assessment of CR (including marrow biopsy) was done after a mean of 2.6 courses (range, two to three). PR was achieved in a further six patients with a mean of three courses (range, two to six). The median duration of CR was 10.1 months and the median duration of PR was 2.4 months. The overall median survival was 14.7 months.

The response to therapy did not differ with age. However, patients < 50 years old had a survival advantage over those > 50 years of age (log-rank test, $P < 0.02$). All complete responders and four of the six partial responders had nodular disease (NPDL Rappaport; cbccf Kiel). This association was not significant. Patients with nodular histology survived significantly longer than those with diffuse histology (log-rank test, $P < 0.05$). In particular, those with DPDL(i) survived for a shorter time than those with NPDL or DWDL.

CR and PR rates for the clinical groups are given in table 1. All patients with remitting and relapsing disease (group 3) achieved a CR, and this association was highly significant (Fisher exact probability

II study of CTX and Pred

| Extranodal disease | Duration of disease (mos) | Prior treatment | No. of courses of CTX and Pred | Response† | Disease-free survival (mos) | Survival as of April 1981 |
|---|---|---|---|---|---|---|
| Liver | 7 | | 1 | D | | 1 |
| | <1 | | 4 | PR | 3 | 20 |
| Liver, ascites effusion | <1 | | 3 | PR | 3 | 31+ |
| | <1 | | 1 | NR | | 5 |
| | <1 | | 3 | D | | 2 |
| Liver | <1 | | 2 | PR | 1 | 23+ |
| | <1 | | 2 | NR | | 6 |
| | | | | | | 6.0§ |
| Liver | 34 | CVP, CB | 3 | D | | 2 |
| | 29 | CVP, CB, Pred | 6 | PR | 5 | 12 |
| Liver | 51 | CB, Pred | 1 | PR | 2 | 22 |
| Retroperitoneum | 39 | CB, CVP | 3 | PR | 4 | 26+ |
| | 19 | CB, CVP | 2 | NR | | 2 |
| Pleural effusion | 4 | CB, Pred, radiotherapy | 1 | NR | | 2 |
| Liver | 6 | CB | 1 | D | | 1 |
| Lung | 16 | CB | 2 | NR | | 14 |
| Liver | 143 | CVP, OPAL,¶ radiotherapy | 2 | NR | | 6 |
| | 42‡ | 2.2 courses‡ | | | | 5.6§ |
| Liver | 13 | CVP, CB | 2 | CR | 10 | 29+ |
| | 54 | CB, CVP, Pred | 2 | CR | 10 | 19 |
| | 29 | CB, CVP | 4 | CR | 13 | 17 |
| | 53 | CB, CVP | 4 | CR | 9 | 36+ |
| | 10 | CTX, Pred, CB | 3 | CR | 13 | 32+ |
| | 24 | CVP, CB | 4 | CR | 10 | 15 |
| | 31‡ | 2.5 courses‡ | | | 10.1§ | 19.3§ |

test, $P < 0.0001$). This group had a survival advantage over the other patients at the $P = 0.06$ level.

### Toxicity

There were four deaths within 1 month of therapy: two of proven sepsis and two sudden deaths at home of suspected sepsis. All patients who died were neutropenic; three had lymphoma present at death. In six patients the intended course of therapy could not be completed because of toxicity: three had life-threatening sepsis, two had CTX-induced cystitis, and one had gastric perforation. In another four patients, steroid doses were reduced: in one patient because of gastrointestinal bleeding and in three because of psychosis. Myelosuppression was severe. In five courses platelet support was required and two patients received granulocyte transfusions. Alopecia, nausea, and mucositis were universal. Hyperglycemia and some degree of mood and sleep disturbance occurred in all patients.

## DISCUSSION

Response was most strongly correlated with the clinical grouping by previous disease behavior. All patients with previously responsive disease achieved CR, similar to the report of Weick and Jones (7). These group 3 patients were younger, but there was no association of response with age in our study. This compares with three PRs in nine refractory patients and three PRs in seven previously untreated patients. The response of the previously untreated group was particularly disappointing but may be in part accounted for on the basis of age and histology. One patient in this group died of septicemia while clinically disease-free and has been classified as a nonresponder.

These results are considerably influenced by the toxicity encountered during therapy. Ten patients (45%) were unable to complete the intended course of therapy because of toxicity, and of these, four died from complications of therapy. Seventeen patients received an adequate trial of at least two courses of treatment, and all who benefited had evident response at the time of initiation of the second course, with all complete responders clinically free of disease after two courses. However, all the complete responders had previously responded to therapy of lesser intensity, to which they may have still been sensitive. In conclusion, therefore, stratification of patients in subsequent trials of therapy for

relapsed non-Hodgkin's lymphoma should include the previous pattern of response as an important prognostic factor. The rapid recurrence of lymphoma between courses and the attendant toxicity of this regimen render it unsuitable as routine therapy for patients with advanced disease. Although the short duration of remission suggests that this approach would not improve treatment at first presentation, it may be of value as a bulk-reducing maneuver in young patients with relapsed follicular lymphoma.

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# Phase II Study of Anguidine in Gastrointestinal Malignancies: A Southwest Oncology Group Study[1,2]

Brief Reports

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## SUMMARY

**The Southwest Oncology Group conducted a phase II study of anguidine in 134 patients with gastrointestinal malignancies. Anguidine was administered as a 4-hour infusion at doses of 3.0 and 4.5 mg/m² daily × 5. Response rates for patients with colon carcinoma were 22% (four of 18 patients without previous chemotherapy) and 6% (four of 63 patients with previous chemotherapy). There were no responses in patients with pancreatic cancer (four patients) or gastric cancer (six). Toxic effects included thrombocytopenia (19.8%), leukopenia (18.8%), nausea and vomiting (49%), hypotension (37%), and confusion (12%). Antitumor activity of anguidine in patients with colon cancer may be similar to that of 5-FU, but nonhematologic toxicity is substantial.**

[Cancer Treat Rep 66:381–383, 1982]

Anguidine is a phytotoxic metabolic product of the plant parasitic fungus *Fusarium equiseti* (1), and was initially selected for phase I trials in man based on its activity against the animal P388 tumor system (2). Subsequently, activity has been demonstrated against 38 colon adenocarcinoma in mice equivalent to that observed with 5-FU.[7] Initial phase I trials of anguidine evaluated a daily × 5 schedule (3,4), and the present phase II trial was based on these data.

## MATERIALS AND METHODS

Patients who had histologically proven gastrointestinal malignancies but who were not candidates for regimens or protocol treatments of higher efficacy or priority were eligible for this study. Eligibility criteria included the following: measurable disease, no prior chemotherapy within the preceding 21 days (42 days with nitrosoureas), no radiotherapy within the preceding 21 days, serum creatinine < 2.0 mg/100 ml, BUN < 30 mg/100 ml, and serum bilirubin < 6.0 mg/100 ml. Patients were divided into good- and poor-risk groups; criteria for the latter group were: age > 65 years, poor tolerance to prior chemotherapy, extensive prior radiation, serum bilirubin between 3.0 and 6.0 mg/100 ml, or hepatic enzyme elevation of greater than three times the institutional normal. A complete response was defined as the disappearance of all clinical evidence of tumor for a minimum of 4 weeks, and a partial response (PR) was defined as a ≥ 50% decrease in the sum of the products of the diameters of all measurable lesions for a minimum of 4 weeks. In patients with liver metastases, a PR was defined as a ≥ 30% reduction in the sum of measurements below the costal margins. Progressive disease was defined as a ≥ 50% increase in the size of any measurable lesion or the appearance of new lesions.

The initial dose and schedule of anguidine were as follows: 4.5 mg/m² daily × 5 for good-risk patients and 3.0 mg/m² daily × 5 for poor-risk patients. Courses were repeated at 28-day intervals if toxicity allowed. The drug was dissolved in 500 ml of 5% dextrose in water and was administered as an iv infusion over 4 hours. Dose levels for escalation or reduction were 1.0, 2.0, 3.0, 4.0, 4.5, 5.0, and 5.5 mg/m². In the absence of toxicity, the dose was increased by one level during the next course. If the granulocyte count nadir was 1000–1999/mm³ and/or the platelet count nadir was 50,000–99,000/mm³, no change in dose level was made. If the granulocyte and platelet count nadirs were 500–999 or 25,000–

[1]Received Mar 10, 1981; revised June 29, 1981; accepted July 9, 1981.

[2]Supported in part by Public Health Service grants CA-04919, CA-22416, CA-16957, CA-12644, CA-03389, CA-12213, CA-21116, CA-03096, CA-20319, CA-27057, CA-16385, CA-04920, CA-13238, CA-13392, CA-04915, CA-14028, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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TABLE 1.—Patient characteristics (Southwest Oncology Group 7735)*

| Characteristic | Colon carcinoma | | Gastric carcinoma | Pancreatic carcinoma | Miscellaneous tumors† |
|---|---|---|---|---|---|
| | Previous chemotherapy | No previous chemotherapy | | | |
| Registered | 90 | 25 | 10 | 7 | 2 |
| Not eligible | 11 | 0 | 2 | 1 | 0 |
| Unevaluable | 8 | 5 | 0 | 1 | 0 |
| Early death‡ | 8 | 2 | 2 | 1 | 0 |
| Evaluable response | 63 | 18 | 6 | 4 | 2 |
| Dose levels | | | | | |
| 3.0 mg/m² | 35 | 9 | 4 | 2 | 1 |
| 4.5 mg/m² | 38 | 9 | 2 | 2 | 1 |
| Median No. of courses received (range) | 2.0 (1–9) | 2.0 (1–14) | 2.0 (1–4) | 1.0 (1–3) | 6,7 |
| Complete response | 0 | 0 | 0 | 0 | 0 |
| PR | 4 (6%) | 4 (22%) | 0 | 0 | 1 (50%) |
| No response | 59 | 14 | 6 | 4 | 0 |

*Unless otherwise specified, values = No. of patients.
†1 patient with metastatic carcinoid, 1 patient with carcinoma of the common bile duct.
‡Patients died < 28 days following start of chemotherapy.

49,000/mm³, respectively, if moderate hypotension (systolic blood pressure, 80–60 mm Hg) occurred, or if grade II CNS toxicity (Southwest Oncology Group criteria) was observed, the dose level was decreased by one level. If more severe toxicity occurred, the dose level was reduced by two levels. All patients had complete physical examinations. Required laboratory studies included a cbc with differential and platelet counts every 7 days and a urinalysis and SMA-12 screen every 28 days. Treatment was continued until there was evidence of progressive disease.

## RESULTS

One hundred and thirty-four patients were registered, 120 were eligible, and 93 were evaluable for response. Table 1 shows their diagnoses, eligibility, and response data. PRs were observed in eight patients with colon cancer and in one patient with carcinoma of the common bile duct. The median duration of response was 3.0 months. Mild myelosuppression in the form of thrombocytopenia (< 100,000 cells/mm³) and leukopenia (< 4000 cells/mm³) occurred in 18.9% and 19.8% of the patients, respectively. Wbc and platelet count nadirs occurred late (Days 19–26) and were 5.5 and 228 × $10^3$/mm³, respectively. Gastrointestinal toxicity in the form of nausea and vomiting was frequent (49% of patients) and was generally moderate to severe. Generally mild hypotension lasting < 2 hours was documented in 37 patients, but in ten patients it was severe, with systolic blood pressures of < 70 mg Hg. Abnormal liver function (bilirubin > 1.5/100 ml or hepatic enzymes more than three times normal) was present in 23 of 37 patients (62%) with hypotension, and in only 27 of 69 (39%) with no hypotension. CNS toxicity in the form of hallucinations and/or confusion occurred in 12% of patients. It was self-limiting and cleared when anguidine was discontinued. Two drug-related deaths occurred, one in a patient with severe myelosuppression who developed sepsis, and one in a patient with severe hypotension.

## DISCUSSION

The toxicity of anguidine was quite varied, with myelosuppression being mild to moderate. The most striking side effects observed were hypotension and CNS toxicity, occurring more commonly in patients with severe liver function abnormalities. The antitumor activity of anguidine was minimal. In patients with previously untreated colon cancer a response rate of 22% was found, and the activity of the drug appears similar to that of 5-FU in a similar group (5). In patients who had had previous chemotherapy, minimal activity was found. The difference in response rates between these two groups reflects the difficulty of evaluating antitumor agents in previously treated patients with gastrointestinal neoplasms. Anguidine used in a daily × 5 schedule appears to have substantial neurologic and cardio-

vascular toxicity and to be of limited value in the treatment of gastrointestinal neoplasms.

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# High-Dose AMSA and Bone Marrow Rescue in Patients With Solid Tumors [1,2]

Brief Reports

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## SUMMARY

**The feasibility of high-dose AMSA followed by autologous bone marrow rescue was studied in seven patients with solid tumors. They received a total of 12 courses of treatment. Total doses ranged from 600 to 1000 mg/m²/course. Major toxic effects were myelosuppression, fever of unknown origin, and stomatitis. One patient with malignant melanoma had stable disease lasting 2 months; the other six had progressive disease.**

[Cancer Treat Rep 66:385–386, 1982]

AMSA is a recently developed antitumor drug that has shown significant activity against leukemias and lymphomas and moderate activity against breast cancer and malignant melanoma (1–4). Its dose-limiting toxic effect is myelosuppression. Nonmyelosuppressive toxic effects consisted of nausea and vomiting. Rare occurrences of neurologic and cardiac rhythm abnormalities have been observed in patients treated with AMSA but did not appear to be dose-related (5). Since the major toxic effect of AMSA appears to be myelosuppression, a study was undertaken to determine the feasibility of using high-dose AMSA followed by autologous bone marrow transplantation in patients with solid tumors.

## MATERIALS AND METHODS

*Patients.*—All patients signed an informed consent statement and were aware of the possible complications of bone marrow aspiration and administration of high-dose AMSA therapy. A total of seven patients received high-dose AMSA therapy with bone marrow rescue; five received two courses of high-dose therapy followed each time by autologous bone marrow infusion. Four patients had malignant melanoma, one had breast cancer, one had histiocytic lymphoma, and one had granulocytic sarcoma. All patients had been treated previously with multiple chemotherapies.

*Evaluation during transplantation.*—During myelosuppression, cbc, differential, and platelet count were obtained daily. SMA-12 and electrolytes were determined three times a week. Chest X-ray and liver, brain, and bone scans were ordered if indicated. Electroencephalogram monitoring was performed during AMSA infusion in five of seven patients. ECG monitoring was done from the first day to the last day of AMSA infusion for five patients. An ECG was obtained on the last day of chemotherapy for all seven patients.

*Chemotherapy administration and dose.*—AMSA was given over 3–5 consecutive days at a total dose of 600–1000 mg/m². The daily dose was dissolved in 500 ml of 5% dextrose and infused over 2 hours.

*Supportive care.*—Four patients were treated in laminar air flow units and received prophylactic nonabsorbable antibiotics as described previously (6). Three patients were treated in an open ward. All patients received prophylactic systemic anti-

[1]Received Apr 9, 1981; revised July 20, 1981; accepted Aug 3, 1981.

[2]Supported in part by Public Health Service grant CA-23077 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Spitzer is a Scholar of the Leukemia Society of America, and Dr. Verma is a Special Fellow of the Leukemia Society of America.

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[5]We thank Ms. Sharon Thomson, Ms. Laura Young, and Ms. Stephanie Goldpin for the preparation of the stored bone marrows, and Ms. Melanie Thomas for typing the manuscript.

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biotic combinations consisting of oral Bactrim and iv tobramycin.

*Characteristics of infused bone marrow.*—The average storage time was 2 months (range, 2 weeks to 6 months) (7). An average of $1.3 \times 10^8$ cells/kg was infused.

## RESULTS

Severe myelosuppression (granulocyte counts $< 0.5 \times 10^9$/liter and platelet counts $< 20 \times 10^9$/liter) was the most consistent side effect in all patients. Stomatitis was the most common gastrointestinal side effect seen in ten courses. Hepatic function abnormalities were seen in one patient with systemic fungus infection and were resolved with antifungal treatment. Gastrointestinal bleeding occurred in one patient who had malignant melanoma with small bowel metastasis. Hematuria occurred in one patient concurrent with thrombocytopenia. Electroencephalogram abnormalities were not found during the nine monitored courses. All patients developed fever during neutropenia. Two patients had documented fungus infection, one local and one systemic. The median duration for neutrophil count suppression $< 0.5 \times 10^9$/liter was 11 days (range, 6–25) and for platelet count suppression $< 20{,}000$/mm$^3$, 7 days (range, 3–15). In most cases, full hematopoietic recovery was obtained within 4 weeks after initiation of AMSA treatment. Three of five patients who received two high-dose treatments had significantly protracted recovery during the second course.

Six patients had progressive disease within 1 month after cessation of AMSA treatment. Two patients had responses, with a $> 50\%$ reduction of measurable tumor for $< 1$ month. One patient had stable disease after the second course of AMSA and received maintenance therapy with AMSA at a dose of 30 mg/m$^2$ for two courses until she had disease progression.

## DISCUSSION

From our data, it appears feasible to give high-dose levels of AMSA safely in patients with solid tumors when bone marrow rescue is given. The dose-limiting toxicity appears to be mucositis at dose levels of about 800–1000 mg/m$^2$. No cardiac or neurologic abnormalities were detected. Previously reported neurologic and cardiac toxic effects from AMSA treatment occurred in patients with other predisposing conditions and did not appear to be dose-related (5).

Dose escalation of AMSA in patients with solid tumors is limited by myelosuppression. Patients with heavily pretreated solid tumors, such as patients with breast cancer, tolerate between 90 and 120 mg/m$^2$, with acceptable toxicity (3). In less intensely pretreated patients, up to 200 mg/m$^2$ can be given (4).

AMSA doses in patients with solid tumors can be escalated four to five times when marrow rescue is utilized. This treatment approach should be investigated in malignancies such as leukemia, breast cancer, and lymphoma which have shown good responsiveness to AMSA in conventional doses (1–3). Autologous bone marrow rescue has been successfully undertaken in patients who received chemotherapy and total-body irradiation (7). In patients treated with high-dose chemotherapy alone, bone marrow transplant enhanced hematopoietic recovery (8). The role of bone marrow transplant after high-dose AMSA needs to be explored, if this therapy should lead to significant response rates in future studies.

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# Relationship of 5′-Nucleotidase Activity and Antileukemic Effect in 2′-Deoxycoformycin Therapy[1,2]

Brief Reports

**Robert L. Wortmann,* John Holcenberg, and David G. Poplack[3]**

2′-Deoxycoformycin (2′DCF), a potent inhibitor of adenosine deaminase (ADA) (EC 3.5.4.4), is currently being investigated in phase I clinical trials for the treatment of lymphoid malignancies. The antineoplastic activity of this drug may result from the accumulation of dATP, which interferes with DNA synthesis by inhibiting ribonucleotide reductase (EC 1.17.4.1) (1,2), and/or from the accumulation of 2′-deoxyadenosine, which inhibits *S*-adenosylhomocysteine hydrolase (SAHH) (EC 3.3.1.1) and thus blocks important methylation reactions (3). In vitro studies have revealed that the combination of 2′-deoxyadenosine and an ADA inhibitor is selectively toxic to acute leukemic lymphoblasts with T cell characteristics (2). Cytotoxicity correlates with dATP accumulation (2). This selectivity may relate to the activity of 5′-nucleotidase (5′NT) (EC 3.1.3.5), which is responsible for degrading deoxyribonucleoside monophosphates (4,5). If deoxynucleotide accumulation is critical to cytotoxicity, then cells with low 5′NT activity would be especially susceptible to ADA inhibition because of their inability to degrade deoxynucleoside monophosphates, and it might be possible to predict response to 2′DCF on the basis of lymphoblast 5′NT activity.

We have investigated the biochemical effects of therapy with 2′DCF on three patients with acute lymphoblastic leukemia (ALL). The results indicate that response to 2′DCF can be achieved despite differences in both immunologic cell type and 5′NT activity.

## MATERIALS AND METHODS

Three patients with ALL refractory to conventional chemotherapeutic agents received 2′DCF at the Milwaukee Children's Hospital as part of a phase I study (table 1). Informed consent was obtained from the patients and their parents. Each 22.5-mg/m$^2$ dose of 2′DCF (supplied by the Investigational Drug Branch, National Cancer Institute, Bethesda, MD) was administered iv in 50 ml of 5% dextrose in water over 30 minutes. Each patient received 100 mg/m$^2$ of allopurinol orally three times a day prior to and during drug therapy.

Intact cell ecto-5′NT activity was quantitated by measuring the conversion of radioisotopic AMP to radiolabeled nucleoside products (5). Dialyzed erythrocyte and mononuclear leukocyte lysates were assayed by previously described radiochemical methods for activities of SAHH and ADA (3,6).

## RESULTS

Lymphoblast ecto-5′NT, ADA, and SAHH activities were determined for each patient on samples obtained prior to the start of therapy (table 1). Ecto-5′NT activities for the two patients with T cell ALL were $< 0.1$ and 0.3 nmol/hr/$10^6$ cells, while a higher value of 32 nmols/hr/$10^6$ cells was observed in the patient with null cell ALL. Pretreatment lymphoblast ADA activities were high in each patient. SAHH activities were elevated in lymphoblasts from each patient (155–203 nmols/hr/mg protein) compared to our values in normal peripheral blood mononuclear cells (69.9 ± 15.5 nmols/hr/mg protein).

Despite the differences in ecto-5′NT activity, each patient had a definite response to therapy (fig 1). Accompanying the decline in absolute lymphoblast counts was a marked inhibition of erythrocyte and lymphoblast ADA and SAHH activity (table 1). Enzyme inhibition occurred sooner for erythrocytes than lymphoblasts, and the percent of ADA inhibition was greater than that for SAHH in each patient.

[1]Received Jan 21, 1981; revised June 3, 1981; accepted July 6, 1981.

[2]Supported in part by grants from the Arthritis Foundation-Wisconsin Chapter; Veterans Administration; and the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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TABLE 1.—Specific activities of enzymes before and after therapy

| Patient No. | Age (yrs) | Surface markers* | 2′DCF dose | Initial lymphoblast ecto-5′NT activity (nmols/hr/$10^6$ cells) | Initial activity (nmols/hr/mg protein) | | | | Time of sample | % of initial activity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | ADA | | SAHH | | | ADA | | SAHH | |
| | | | | | Rbc | Wbc | Rbc | Wbc | | Rbc | Wbc | Rbc | Wbc |
| 1 | 19 | T+,E−,SIg−,ALL− | Daily for 3 consecutive days | 0.3 | — | 12,150 | — | 165 | 24 hrs after dose 1 | — | 1 | — | 2 |
| | | | | | | | | | 24 hrs after dose 3 | — | 2.4 | — | 6 |
| | | | | | | | | | 1 wk after dose 3 | — | 17 | — | 59 |
| 2 | 14 | T+,E+,SIg−,ALL− | Daily for 3 consecutive days | < 0.1 | 180 | 21,350 | 5.0 | 203 | 6 hrs after dose 1 | 1 | 21 | 10 | 8 |
| | | | | | | | | | 24 hrs after dose 1 | 0.3 | 1.4 | 3 | 12 |
| | | | | | | | | | 24 hrs after dose 2 | 1 | 1.2 | 2 | 2.5 |
| 3 | 13 | T−,E−,SIg−,ALL− | 1 dose every 4 days | 32.0 | 155 | 18,184 | 3.0 | 155 | 72 hrs after dose 1 | 0.6 | 1.8 | 13 | 30 |
| | | | | | | | | | 96 hrs after dose 1 | 0.6 | 2.3 | 13 | 14 |
| | | | | | | | | | 6 hrs after dose 2 | 0 | 1.8 | 6 | 16 |

*Lymphoblasts were characterized by reaction with antibodies against immunoglobulins (SIg), thymocytes (T), and common ALL antigen (ALL), and by formation of rosettes with sheep erythrocytes (E) (10,11).

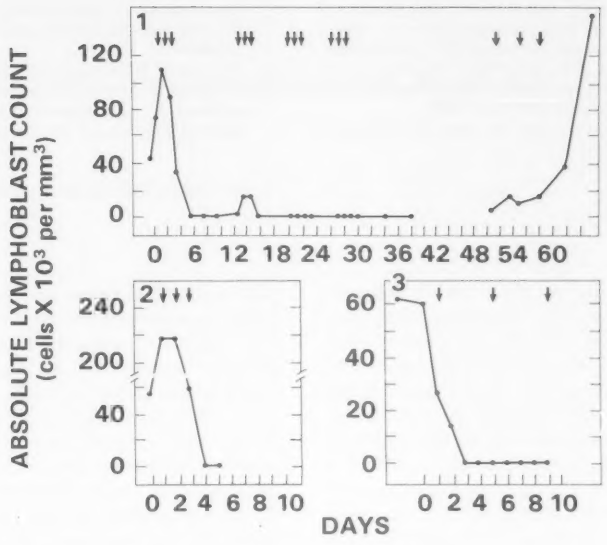


FIGURE 1.—Response to 2′DCF in patients 1–3. Each arrow represents a single dose of medication (22.5 mg/m²).

## DISCUSSION

Despite differences in leukemia cell type and ecto-5′NT activity, each of our three patients experienced a profound decrease in the number of circulating leukemia cells following administration of 2′DCF. In each case the decline was associated with major inhibition of ADA and SAHH activity in both peripheral mononuclear cells and erythrocytes. These results provide clinical correlation with the recent in vitro observations that null lymphoblasts are susceptible to ADA inhibition despite higher ecto-5′NT activity (7). These data suggest that either ecto-5′NT does not regulate the cytotoxicity of ADA inhibition or that in vivo, 2′DCF causes changes in metabolism that exceed the capacity of null cell 5′NT to degrade deoxynucleoside monophosphates. Even though null lymphoblasts have ecto-5′NT activities 100-fold greater than T lymphoblasts, this is still a low specific activity compared to that found in other cells. Fibroblasts, for example, have ecto-5′NT activity 900-fold greater than null lymphoblasts (8).

The clinical responses observed in this and other studies suggest that 2′DCF is a promising agent for treatment of lymphoid malignancies. These data do not define whether dATP accumulation or SAHH inhibition mediates the lysis of peripheral lymphoblasts. Erythrocyte and lymphoblast SAHH activities were decreased in each of our patients. SAHH inhibition has also been reported in a patient who responded to 2′DCF with complete leukemic cell lysis (9). These results suggest that ecto-5′NT activity alone may not be useful in predicting which patients will respond to 2′DCF.

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# Chemoimmunotherapy for Autochthonous Acute Rat Leukemias[1]

Brief Reports

W. J. Zeller,* K. Reusch, and D. Schmähl[2,3]

## SUMMARY

**Eighty autochthonous acute rat leukemias that had been induced by ethylnitrosourea were used to compare the effect of single-drug therapy with vincristine, doxorubicin, or cytarabine with the effect of combination therapy using these three drugs with or without BCG-Pasteur F. The combination chemotherapy was clearly superior to the single-drug therapy. The best results (survival time; duration of remission) were achieved by vincristine, doxorubicin, and cytarabine plus BCG, although compared to the combinations without BCG, these results were not significant.**

[Cancer Treat Rep 66:391–394, 1982]

The possibility of achieving high response rates and extended durations of remission in human leukemias with established treatment schedules increases the difficulty of clinically evaluating new drugs against this disease. Therefore, it is essential to look for new models which are similar to the human situation. In a previous paper we showed that autochthonous acute rat leukemias induced by ethylnitrosourea (ENU) demonstrate parallels to human acute leukemia and might be valuable for the improvement of a clinically established therapy schedule (1). We reported on the response of autochthonous acute ENU-induced leukemias to a combination of vincristine (VCR), doxorubicin (ADR), and cytarabine (Ara-C) (1). The present investigation compares the effect of single-drug therapy with VCR, ADR, or Ara-C with the effect of combination therapy with these agents against such acute leukemias. Studies on L1210 leukemia (2), which had shown that the combination of daunorubicin plus Ara-C had a synergistic effect if daunorubicin was applied after Ara-C, suggested an investigation of two variations of the combination of VCR, ADR, and Ara-C. Results from an active unspecific immunotherapy for adult acute leukemia using BCG (3) suggested an additional application of BCG-Pasteur F.

## MATERIALS AND METHODS

### Animals and Leukemias

The leukemias were induced by long-term iv application of ENU to 3-month-old male Wistar rats (breed: Ivanovas, Kisslegg, Federal Republic of Germany). The method of induction as well as the diagnosis of leukemia has been described (1). A total of 80 exclusively immature-cell leukemias were randomly divided into seven experimental groups (table 1). Seventy-two leukemias developed after a weekly dose of 15 mg/kg of ENU for 15 weeks and eight developed after a weekly dose of 75 mg/kg of ENU for 3 weeks; of these latter eight leukemias, four were distributed to group 2, two to group 6, and one each to groups 4 and 7.

The median wbc count for all groups was > 100,000/mm$^3$. Only six of the 80 leukemias produced a peripheral wbc count < 50,000/mm$^3$. The proportion of blast cells in the peripheral blood was high (usually > 80%). In the peripheral blood only isolated mitoses were observed (mitotic index less than one per thousand), while in the spleen the mitotic index was about five per thousand. All animals had pronounced hepatosplenomegaly; 16 animals (20%) also had enlarged lymph nodes at the time of diagnosis. As we demonstrated in a preceding paper, animals with distinctly reduced Hgb and/or platelet counts have only a small chance of surviving the first weeks of treatment (1). Thus, only animals in good general condition were included in the present investiga-

[1]Received Oct 27, 1980; revised June 29, 1981; accepted July 20, 1981.

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[3]We thank Pia Guckert and Christel Kamphausen for skillful technical assistance; Drs. K. Goerttler and D. Komitowski for histologic examinations; and G. Rosenkranz for assistance in the statistical analysis.

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TABLE 1.—Chemoimmunotherapy for autochthonous acute rate leukemias

| Group No. | No. of animals | Drugs | Treatment schedule* | Single dose (mg/kg) | Median total dose in mg/kg (range) | Median duration of remission† in days (range) | Median survival time‡ in days (range) | % increase in lifespan |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | VCR | Once weekly | 0.15 | 0.75 (0.3–1.05) | 4 (4–22) | 43 (14–70) | 219 |
| 2 | 10 | ADR | Once weekly | 1.5 | 6.0 (3.0–9.0) | 10 (2–52) | 31 (10–57) | 130 |
| 3 | 10 | Ara-C | Twice daily × 5 days/wk | 10 | 820 (510–1110) | 33 (8–64) | 59 (37–84) | 337 |
| 4 | 15 | VCR | Once weekly | 0.1 | 0.4 (0.3–0.4) | 60 (15–173) | 80 (42–173) | 530 |
| | | ADR | Once weekly§ | 1.0 | 4 (3–4) | | | |
| | | Ara-C | Twice daily × 5 days/wk | 10 | 490 (260–1140) | | | |
| 5 | 15 | VCR | Once weekly | 0.1 | 0.4 (0.3–0.4) | 67 (15–108) | 91 (40–113) | 574 |
| | | ADR | Once weekly§ | 1.0 | 4 (3–6) | | | |
| | | Ara-C | Twice daily × 5 days/wk | 10 | 460 (260–700) | | | |
| | | BCG | Twice weekly | 7.5 mg/rat | 45 (30–60) mg/rat | | | |
| 6 | 10 | VCR | Once weekly | 0.1 | 0.4 (0.2–0.4) | 42 (8–81) | 67 (29–115) | 396 |
| | | ADR | Once weekly¶ | 1.0 | 4.0 (1.0–4.0) | | | |
| | | Ara-C | Twice daily × 4 days/wk | 12.5 | 425 (140–880) | | | |
| 7 | 10 | Control | | | | — | 13.5 (5–39) | — |

*Outline of timing given in fig 1; all treatment was given ip except ADR (iv) and BCG (sc).

†Complete regression of all visible and palpable signs of leukemia (enlargement of liver, spleen, and lymph nodes; peripheral blast cell count).

‡A total of 12 rats died during remission (histologically verified): 1 rat in group 2 after 18 days; 4 in group 4 after 42, 58, 108, and 173 days; 6 in group 5 after 57, 64, 78, 81, 88, and 108 days; and 1 in group 6 after 55 days. The remaining animals died of leukemia after relapse.

§ADR within 1 hr after VCR.

¶ADR 1 day after Ara-C.

tion. For groups 1–7, Hgb (median range, 12.1–12.8 g/100 ml) and platelet counts (median range, 200,000–395,000/mm$^3$) were approximately comparable. Similarly, the manifestation times were without significant difference.

## Drugs and Treatment

VCR (Vincristin-Lilly, Eli-Lilly, Geiben, Federal Republic of Germany) was injected in a 0.02% solution in water, ADR (Adriblastin, Farmitalia, Freiburg, Federal Republic of Germany) was injected in a 0.2% solution in physiologic saline, and Ara-C (Alexan, Mack, Illertissen, Federal Republic of Germany) was given in a 2% solution in sodium lactate-buffered physiologic saline. BCG-Pasteur F (75 mg of BCG/ml) was obtained from the Institut Pasteur, Paris, France.

Table 1 gives the individual treatment schedules. In groups 4 and 5, VCR and ADR were given together 2 ½ days before the start of treatment with Ara-C (fig 1, D and E), and in group 6 only, VCR was applied before Ara-C while ADR was given about 20 hours after Ara-C (fig 1F). The total dose for all groups was approximately equitoxic (≤ LD10). The maximal tolerated doses have been described in preceding experiments (1).

## Statistical Evaluation

Since no reasons for the assumption of a particular survival time distribution were available, distribution-free methods were used for data analysis.

Survival time as well as the duration of remission was compared by the Breslow test (4), followed by multiple comparisons according to the sequential method of Holm (5).

## RESULTS AND DISCUSSION

Survival times of treated rats and untreated controls are given in table 1. Survival times of all groups except group 2 differed significantly from untreated controls. Furthermore, group 2 differed significantly from group 3, group 5 from groups 1–3, group 4 from groups 1 and 2, and group 6 from group 2 (multiple significance level, $\alpha \leq 0.05$). As shown in table 1, the survival time correlates with the duration of remission. With regard to duration of re-

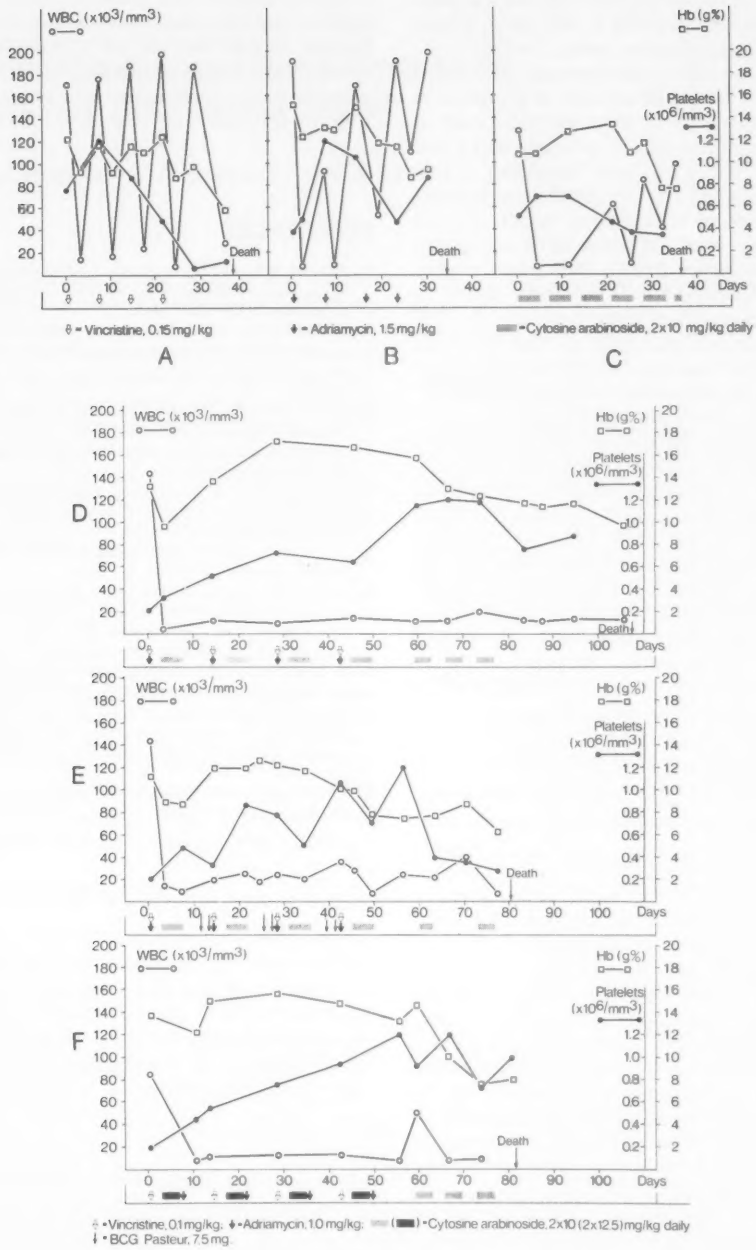


FIGURE 1A–F.—Examples of single-drug and combination chemotherapy for acute rat leukemias (A–F each represents the course of 1 animal). Note short-term reduction of wbc count after single-drug chemotherapy and superiority of combination chemotherapy with increase in Hgb and platelet counts during treatment (remissions).

mission, group 1 differed significantly from groups 3–6, group 2 from groups 4 and 5, and group 3 from group 5 (multiple significance level, $\alpha \leq 0.05$).

Six typical courses (individual animals) are shown in figure 1 (A–F). Of the 40 animals in groups 4–6, nine (~ 22%) survived > 90 days after the start of treatment (four animals each in groups 4 and 5 and one in group 6). Three of these "long-term survivors" died in remission. In contrast to experimental data (2) and in accord with clinical results (6), no improvement was observed when ADR was given after rather than before Ara-C. This result might, of course, be influenced by a synergistic action of VCR and ADR when given simultaneously (7). The combination treatment plus BCG yielded the longest median survival time and duration of remission. Compared to the combinations without BCG, however, these results were not significant.

More recent studies on the kinetics of these autochthonous acute rat leukemias revealed some further similarities to the human situation. In five animals, the labeling index ranged between 0.1% and 5% (median, 2.5%) in the blood, between 7.3% and 13.9% (median, 8.4%) in the marrow, between 8.3 and 25% (median, 18%) in the spleen, and between 5% and 28% (median, 21%) in the liver; the S-phase duration of leukemic blast cells in the spleen ranged between 20 and 118 hours (K. Reusch and W. J. Zeller, unpublished data). When these data are compared with those from transplanted rat leukemias and with those from human acute leukemias (8,9), it is obvious that autochthonous acute rat leukemias are closer to human acute leukemias than are transplant models.

On the whole, the present study confirms our opinion that chemically induced autochthonous leukemias in rats may be an appropriate predictive system for human acute leukemias and could be suitable for investigating and perhaps complementing therapy schedules already used in the clinic.

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# Comparative Cardiac and Renal Toxicity of Daunorubicin in the Rat and Rabbit [1]

Brief Reports

D. Deprez-DeCampeneere,[2] R. Jaenke,[3,*] and A. Trouet[2]

## SUMMARY

**A direct comparison of the cardiac and extracardiac effects of repeated daunorubicin administration in the rat and rabbit demonstrated a basic similarity in the cardiac myocyte response. The rat, however, appears to develop a more severe drug-related nephropathy, resulting in widespread soft tissue mineralization that includes vessels and the myocardium. These findings suggest that the severity and development of acute and chronic anthracycline-induced cardiac lesions may, in the rat, be related or dependent, in part, on concomitant nephrotoxicity.**

[Cancer Treat Rep 66:395–397, 1982]

Investigation of chronic anthracycline-induced cardiomyopathy (CMY) requires an appropriate animal model for the screening of newly developed analogs and derivatives for cardiotoxicity and for the study of pathogenetic mechanisms of CMY. The present study was conducted to compare the chronic cardiac and renal responses of the rat and rabbit to the anthracycline antibiotic daunorubicin (DNR).

## MATERIALS AND METHODS

Twenty male CDF Fischer and 20 Sprague-Dawley rats received 2.0 mg/kg of DNR iv once weekly while nine male Belgium white rabbits received 1.3 mg/kg of DNR iv twice weekly. These treatment doses represented the maximal dose failing to produce fatal, acute hematopoietic or gastrointestinal toxicity. Ten rats and six rabbits served as non-drug-treated controls. Histopathologic examination and evaluation of tissues were done as described previously (1).

## RESULTS

All DNR-treated rabbits survived a scheduled 11-week treatment period with no clinical evidence of severe acute toxicity and with weight gain rates similar to controls. At slightly lower weekly treatment doses, 35 of 40 rats survived 11 weeks of treatment but only one of 40 DNR-treated rats survived the scheduled 14-week treatment period. After 4 weeks, weight gains of treated rats were reduced sharply compared to controls.

### Cardiac Lesions

Congestive heart failure was noted in five of nine treated rabbits and in 14 of 40 treated rats. The hearts of DNR-treated rabbits, although of normal size, exhibited extensive gross fibrosis. Hearts from drug-treated rats were small and pale but not grossly fibrotic.

Microscopic CMY was present in all drug-treated rabbits, with 67% showing severe diffuse lesions similar to those described previously (1,2), ie, early myocyte vacuolation followed by loss of myofilaments and myocyte atrophy and lysis with noninflammatory fibrosis. Myocardial mineralization was absent. Although widespread, CMY in the rabbit was most severe at the base of the heart and in the subendocardial musculature and papillary muscles of the left heart and septum.

Although the basic character and incidence (29 of 40 rats) of CMY were similar in the rat and rabbit, rats consistently exhibited left atrial lesions and, less frequently, CMY of the right ventricle. Also, in the rat, CMY was frequently multifocal in distribution with perivascular lesions of coronary arteries.

Severe and often diffuse cardiac myofiber mineralization was common in DNR-treated rats. Rarely observed in the rabbit, this lesion in the rat was

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sometimes associated with intimal and medial arterial mineralization. In contrast to the noninflammatory nature of the rabbit CMY, histiocytic proliferation was common in the rat, which also showed prominent interstitial edema and myofiber atrophy but little fibrosis.

### Renal Lesions

Although rabbits in this study exhibited mild deep cortical glomerular lesions, rats consistently showed severe diffuse glomerular and tubular changes. These lesions were characterized by hypocellular swollen glomeruli with expanded eosinophilic mesangial regions, compressed capillary lumina, and foamy peripheral epithelial cells. Dilated cortical and medullary tubules contained eosinophilic casts with atrophic epithelium and were surrounded by thickened basement membranes and foci of mineralization. Interstitial edema and mild diffuse fibrosis were often present.

Accompanying and frequently paralleling the severity of the renal lesions in the rat was severe mineralization of the stomach, pulmonary parenchyma, aorta, and splenic and intestinal arteries, as well as that described above in the myocardium.

## DISCUSSION

The results of this study indicate the similarity of the myocardial light microscopic histopathologic changes observed in the rat and rabbit after chronic anthracycline administration sufficient to cause advanced CMY and congestive heart failure. Mettler et al (3) have shown that at the ultrastructural level, alterations in myocyte subcellular components are also similar, suggesting a common basic mechanism of anthracycline action in the two species. In both animal models the morphologic expressions of myocardial lesions are delayed and their severity appears to be dose-related. The principal differences observed between the rat and rabbit appear to be related to the distribution of myocardial changes and the severity of the accompanying extramyocytic reaction.

Severe renal lesions were consistently observed in the DNR-treated rats in this study. Mettler et al (3) also reported renal changes in repeatedly treated rats but no evidence of secondary tissue mineralization was reported. Sternberg et al (4), however, reported a high incidence of chronic glomerulonephritis associated with secondary hyperparathyroidism, metastatic calcification of the kidneys and stomach, and hyperosteotic bone disease 6 months after a single high dose (10 mg/kg) of DNR, indicating that the renal effects of the anthracyclines are immediate and potentially nonreversible in the rat.

Fajardo et al (5) have recently described in detail renal lesions in rabbits treated repeatedly with doxorubicin. The lesions were morphologically similar to those observed in the rat in the present study. Although the doxorubicin-induced lesions in the rabbit appeared to be of a severity similar to those described by Mettler et al (3) in rats receiving doxorubicin, the rat renal lesions induced by DNR in the present study were strikingly more severe than those produced by doxorubicin in either the rat (3) or rabbit. The existence of obvious species-related variations in renal and cardiac toxicity to the various anthracyclines has been suggested previously (2).

A striking finding in the rats of this study was the presence of widespread soft tissue mineralization, which is related at least in part to the severe renal lesions observed in this species. Other studies in the rat have demonstrated hypocalcemia 6–24 hours after a single drug injection (6) or terminally after repeated drug injections (3). One of these studies (6) also reported increased total calcium content in the ventricular myocardium. Similar myocardial calcium results have been reported in the rabbit (1,7); however, none of these studies have reported microscopic evidence of myocardial or other soft tissue mineralization such as was noted in rats of this study. The deleterious effects of increased intracellular calcium concentrations on the myocardium are well-documented (8), and it has been suggested (9) that anthracycline-induced alterations in myocyte membrane permeability to calcium may contribute to or be responsible for the drug-induced CMY characteristic of this class of compounds.

It appears that the myocardial calcification observed may be related to a variety of factors that contribute in a complex manner to the pathogenesis of the anthracycline-induced CMY. Results in the rat (4) demonstrate that severe myocardial mineralization in anthracycline-treated animals may be metastatic in nature and related to concomitant severe renal damage. However, studies in the rabbit (1,6) appear to indicate that increased myocardial calcium levels also occur in the absence of severe renal disease and may thus be dystrophic in nature and related to primary anthracycline-induced myofiber damage or, as suggested by Young (9), directly related to drug-induced alterations in calcium metabolism and direct myocyte damage by this cation. The potential for this complex form of myocardial damage must be recognized in the testing for cardiotoxic potential of anthracycline derivatives and analogs in the rat and in comparing the CMY produced by a particular compound in the rat

and rabbit. In addition, early ECG changes in rats used in the detection of potential cardiotoxicity of anthracycline antibiotics should be evaluated carefully in light of the immediate and delayed effects of these compounds on serum and myocardial calcium concentrations secondary to renal damage and the possible effects of these changes on the electrical conductivity of the heart (10). Changes in the ECG may be secondary to altered serum or tissue calcium levels, which may or may not be important in the pathogenesis of chronic anthracycline-induced CMY. The changes in calcium levels and the ECG may be secondary to renal damage and thus dependent on the severity of kidney lesions induced by a specific compound, rather than being dependent on relative degrees of chronic cardiotoxicity. Studies in our laboratory have in fact shown significant comparative differences in the degree of renal change produced by a variety of anthracycline antibiotics (unpublished data).

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# Evaluation of Azapicyl in Soft Tissue Sarcomas in Children: A Report From the Children's Cancer Study Group [1,2]

Clinical Trials Summaries

Ruth Heyn,[3] William Krivit,[4] Harland Sather,[5] and Denman Hammond [5,*]

New effective agents for rhabdomyosarcoma (RMS) in children have been few. Azapicyl (1-acetyl-2-picolinoylhydrazine) is a synthetic compound whose mechanism of action is unknown (D. D. Von Hoff, personal communication). Limited activity with this agent has been reported in several adult tumors (1). In human trials, a twice daily oral dose was as effective as various dose schedules administered iv (2). In adults, the drug dose has been limited by CNS toxicity. Prior studies included complete remissions in two patients with resistant RMS, lasting 6 weeks and 6 months, respectively (3,4). These responses prompted a further clinical trial with this agent by the Children's Cancer Study Group.

Twenty-two children with resistant RMS or undifferentiated sarcoma were treated with azapicyl following physical and neurologic examination, blood cell counts, tests of renal and liver functions, bone marrow examination, and appropriate X-ray studies. Azapicyl was initially administered at a dose of 350 mg/m²/day orally for 14 days, with an escalation to 400 mg/m²/day at 14 days and another to 450 mg/m²/day at 28 days if disease was static or had begun to respond at these intervals. An adequate trial period was 28 days. Followup studies were repeated at 2-week intervals.

Seventeen children with RMS and five with undifferentiated sarcoma were entered in the study. Table 1 (page 400) presents the pre-study characteristics of the patients, their response to therapy, and the drug toxicity. There were no measurable responses in ten adequately treated patients. Twelve patients had at least one escalation in drug dose, and two had a second increase. Only two patients had therapy interrupted because of toxic effects. Five children had somnolence but no other CNS toxic effect.

Although the dose of azapicyl used in this study was greater than the dose associated with a previously reported complete remission in a child with RMS (3), toxicity was minimal. Since maximally tolerated doses were not reached in most patients, higher doses of drug should be considered if further clinical trials with this agent are pursued.

[1]Received June 1, 1981; revised Sept 8, 1981; accepted Sept 21, 1981.

[2]Supported by Public Health Service grants (*see* Appendix, page 401) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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TABLE 1.—Patient characteristics, treatment, and response to azapicyl

| Patient No. | Sex | Age (yrs) | Primary site | Disease when entered in study | Days treated | No. of drug escalations | Response* | Toxicity |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Rhabdomyosarcoma*** | | | | |
| 1 | F | 5 | Left foot | Right lung, right thigh | 57 | 2 | PD | Somnolence |
| 2 | F | 10 | Right chest wall | Bilateral lungs, chest wall, hilar mass | 14 | 0 | PD (IT) | |
| 3 | M | 5 | Right middle ear | Right external canal and mastoid | 43 | 2 | PD | |
| 4 | M | 9 | Left groin | Abdomen, testes, bilateral lungs, mediastinum | 34 | 1 | PD | |
| 5 | F | 13 | Left breast | Breast masses, + cerebrospinal fluid, axilla, back | 32 | 1 | PD | Rash, somnolence |
| 6 | F | 5 | Left middle ear | Bilateral lungs, left middle ear | 28 | 1 | PD | |
| 7 | F | 11 | Post mediastinum | Bilateral lungs | 20 | 1 | PD (IT) | Nausea and vomiting |
| 8 | F | 16 | Left foot | Bone marrow, paraspinal mass, bone | 28 | 0 | PD | Somnolence |
| 9 | M | 13 | Left foot | Right lung | 29 | 1 | PD | |
| 10 | M | 13 | Pelvis | Bone marrow, bilateral lungs, bone, periumbilical mass | 22 | 1 | PD (IT)† | Hematologic |
| 11 | F | 8 | Nasopharynx | Nasopharynx, brain | 20 | 0 | PD (IT)† | |
| 12 | M | 6 | Left foot | Multiple bones, bone marrow, liver, lung | 17 | 0 | PD (IT) | ↑ BUN, nausea and vomiting, rash, somnolence, hematologic |
| 13 | M | 17 | Right upper arm | Testes, skin, bilateral pleural effusions | 11 | 0 | PD (IT)† | |
| 14 | F | 3 | Soft palate | Bilateral lungs, neck, palate, bones | 7 | 0 | PD (IT)† | |
| 15 | F | 8 | Anterior neck | Mediastinum, post-tracheal mass | 20 | 0 | PD (IT) | Nausea and vomiting |
| 16 | M | 2 | Base of tongue | Cervical mass | 57 | 1 | NR | |
| 17 | M | 16 | Neck | Bilateral lungs, bone marrow, retro-orbital mass, neck | 9 | 0 | PD (IT)† | |
| | | | | ***Undifferentiated sarcoma*** | | | | |
| 18 | M | 11 | Left buttock | Buttock and pelvis | 35 | 1 | PD | |
| 19 | M | 13 | Right pleura | Brain, right lung | 28 | 1 | PD | Somnolence |
| 20 | F | 5 | Right upper lid | Right facial mass, right mandible | 23 | 1 | PD (IT)† | |
| 21 | M | 15 | Nasopharynx | Brain | 14 | 0 | PD (IT) | Somnolence |
| 22 | F | 21 | Base of tongue | Bilateral lungs | 9 | 0 | PD (IT)† | |

*PD = progressive disease, NR = no response, IT = inadequate trial. At the doses used, no responses were achieved.
†Death before 28 days.

## APPENDIX

### Principal Investigators in the Children's Cancer Study Group

| *Institution* | *Investigator* | *Grant No.* |
|---|---|---|
| Group Operations Office, University of Southern California Comprehensive Cancer Center, Los Angeles | Denman Hammond<br>Richard Honour<br>Harland Sather<br>Richard Sposto<br>John Weiner | CA-13539 |
| University of Michigan Medical Center, Ann Arbor | Ruth Heyn | CA-02971 |
| Children's Hospital National Medical Center, George Washington University, Washington, DC | Sanford Leikin | CA-03888 |
| Memorial Sloan-Kettering Cancer Center-Cornell University, New York, NY | Denis R. Miller | CA-14557 |
| Children's Hospital of Los Angeles, University of Southern California | Gussie Higgins | CA-02649 |
| Children's Hospital of Pittsburgh, University of Pittsburgh, PA | Vincent Albo | CA-07439 |
| Children's Hospital of Columbus, Ohio State University | William Newton | CA-03750 |
| Children's Orthopedic Hospital and Medical Center, University of Washington, Seattle | Ronald Chard | CA-10382 |
| University of Minnesota Health Sciences Center, Minneapolis | Mark Nesbit | CA-07306 |
| Children's Memorial Hospital, Northwestern University, Chicago, IL | Helen Maurer | CA-07431 |
| Children's Hospital of Philadelphia, University of Pennsylvania | Audrey Evans | CA-11796 |
| James Whitcomb Riley Hospital for Children, Indiana University, Indianapolis | Robert Baehner | CA-13809 |
| University of Iowa, Hospitals and Clinics, Iowa City | C. Thomas Kisker | CA-29314 |

# Phase II Trial of AMSA in Refractory Breast Cancer[1]

**Mario De Lena, Anna Rossi, and Gianni Bonadonna**[2,*]

Clinical Trials Summaries

AMSA is an acridine derivative that has recently entered phase II evaluation (1). Initial favorable results in advanced metastatic breast cancer (2,3) stimulated the present study, aimed at confirming the activity of the drug and meeting the clinical need for new effective agents for the treatment of this disease.

## PATIENTS AND METHODS

Thirty-three women with metastatic breast cancer refractory to one or more chemotherapeutic regimens were treated with AMSA between February and May 1980. Twenty-seven patients fulfilled the criteria of eligibility for the study, ie, measurable parameters for drug response and adequate duration of therapy (minimum of two cycles unless clear disease progression was documented after first cycle). The main patient characteristics are summarized in table 1. Although all patients received previous chemotherapy, in five women AMSA represented their first systemic treatment for relapse after adjuvant chemotherapy. All but two patients were ambulatory; five additional patients were treated as inpatients only because of concomitant palliative radiotherapy.

AMSA was administered as a 30-minute infusion at single doses of 90 and 120 mg/m$^2$, every 3–4 weeks. The lower dose level was assigned to patients with widespread bone involvement and/or previous extensive irradiation or limited bone marrow reserve. Standard criteria for response assessment were utilized. The median number of cycles administered was three (range, one to nine).

TABLE 1.—Patient characteristics and treatment results

| Characteristic | No. of patients |
|---|---|
| Total evaluable | 27 |
| Age (yrs) | |
| Median: 53 | |
| Range: 34–70 | |
| Previous chemotherapy* | 27 |
| CMF | 3 |
| CMF → AV | 13 |
| CMF → AV → phase II drugs | 11 |
| Previous endocrine therapy | 21 |
| Previous radiotherapy | 8 |
| Limited | 6 |
| Extensive† | 2 |
| Performance status (Karnofsky) | |
| ≥ 70 | 15 |
| 60–50 | 10 |
| < 50 | 2 |
| Dominant site of disease | |
| Soft tissue | 10 |
| Bone | 4 |
| Viscera | 13 |
| Dose level (mg/m$^2$) | |
| 90 | 9 |
| 120 | 18 |
| Response, ie, objective improvement | 2 (7%) |
| Toxicity | |
| Nausea, vomiting | 11 |
| Skin rash | 1 |
| Leukopenia (cells/mm$^3$) | |
| 3900–2500 | 12 (43%) |
| < 2500 | 7 (26%) |
| Thrombocytopenia | 0 |

*C = cyclophosphamide; M = methotrexate; F = 5-FU; A = doxorubicin; and V = vincristine. In 5 patients (1 CMF, 4 CMF→AV), previous chemotherapy was given as adjuvant to mastectomy.

†≥ 50% of marrow volume.

## RESULTS

None of 27 evaluable patients showed complete or partial responses. Two women with soft tissue and liver metastases experienced minimal objective

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tumor regression for periods of 9 and 3 months, respectively. The transient regression of metastatic hepatomegaly was assessed through physical examination only. Both patients received the drug at a dose of 120 mg/m$^2$. Previous chemotherapy consisted of CMF and AV in the patient with soft tissue disease and CMF in the patient with liver metastases.

Toxic effects are summarized in table 1. Nausea and vomiting were mild. Myelosuppression was evaluated in outpatients on the basis of a complete hemogram on Day 22 of each cycle. Median leukocyte count nadir, evaluated in seven patients, was 1200/mm$^3$ (range, 400–2200) and occurred on Day 8 (range, 7–11). No serious infections were associated with severe neutropenia. Thrombocytopenia was not observed.

In one of the patients showing minor response, reversible congestive heart failure occurred after nine cycles of AMSA (total dose, 840 mg/m$^2$). The patient had previously been treated with doxorubicin (total dose, 420 mg/m$^2$) and mediastinal irradiation 36 months before and had a normal ECG at the start of AMSA treatment. Drug-induced cardiomyopathy possibly related also to previous treatments cannot be excluded in this patient (4).

Present data failed to confirm a significant activity of AMSA in patients with advanced breast cancer refractory to conventional polychemotherapy. Minimal subjective toxicity and moderate myelosuppression were also confirmed in this group of previously treated women. Prior multiple-drug treatments as well as the dose schedule employed might have had an impact on our negative results. However, on this basis, a trial with AMSA in untreated patients does not seem warranted.

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# Phase II Evaluation of VM-26 in Patients With Metastatic Transitional Cell Carcinoma of the Urinary Tract: An Eastern Cooperative Oncology Group Study[1,2]

Clinical Trials Summaries

**Raman Qazi,* Paul Elson, and Janardan D. Khandekar[2,3]**

VM-26 is a semisynthetic podophyllotoxin derivative that has demonstrated significant activity in Hodgkin's and non-Hodgkin's lymphoma (1) and in various pediatric malignancies (2). Because of reports of objective responses to VM-26 in phase I studies in bladder carcinoma (3,4), the present phase II study was initiated.

## MATERIALS AND METHODS

Forty patients (26 males and 14 females) with histologically confirmed disseminated transitional cell carcinoma of the urinary tract were entered in this study. All patients had objectively measurable or evaluable disease, a wbc count > 4000/mm$^3$, a platelet count > 100,000/mm$^3$, and an Eastern Cooperative Oncology Group performance status of 3. The median age of the patients was 57 years (range, 52–75). Eight patients had received no prior therapy, seven had been treated with radiation therapy, 17 had been treated with chemotherapy, and eight had had both radiation therapy and chemotherapy. None of the patients had previously received VM-26.

VM-26 was given at an initial dose of 30 mg/m$^2$ in 100 ml of 0.9% saline infused over 1 hour daily × 5 every 3 weeks. Patients > 65 years of age and patients who had had prior radiation therapy received a 20-mg/m$^2$ infusion daily × 5 every 3 weeks. If, after this initial dose, the patient's wbc and platelet count nadirs were > 3000/mm$^3$ and > 100,000/mm$^3$, respectively, and if, prior to the second course, the wbc count was > 4000/mm$^3$, the dose of VM-26 was increased to 30 mg/m$^2$. In patients who had a bilirubin > 3.0 mg/dl or SGOT > 300 IU/ml, the dose of VM-26 was decreased by 50% until these levels returned to normal. If vomiting, diarrhea, or stomatitis were severe, the next cycle of chemotherapy was delayed until toxic effects had disappeared, and the dose was reduced by 25%. No chemotherapy was given if the wbc count was < 3000/mm$^3$ or the platelet count was < 75,000/mm$^3$. Twenty-seven patients received two or more cycles of chemotherapy, while 13 patients received only one cycle because of disease progression (ten patients), worsening performance status (two), and wbc count < 3000/mm$^3$ (one). Eight patients received doses that were lower than planned, because of either advanced age and liver disease (two patients) or low wbc count (six).

Partial response was defined as a 50% regression of all measurable disease as determined by the sum of the areas of the lesions. For patients with liver metastasis, a PR was defined as a ≥ 30% reduction in the extension of liver beyond the costal margin. Disease progression was defined as a 25% increase in the dimensions of measurable disease or development of new lesions.

[1]Received July 6, 1981; revised Oct 5, 1981; accepted Oct 19, 1981.

[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone, chairman*; CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: University of Rochester Cancer Center, NY (*R. Qazi*, CA-11083); Sidney Farber Cancer Institute, Boston, MA (*P. Elson*, CA-23318); and Northwestern University Medical Center, Chicago, IL (*J. Khandekar*, CA-17145). Other participating institutions include: Albany Medical College, NY (CA-06594); Albert Einstein College of Medicine, Bronx, NY (CA-14958); American Oncologic Hospital, Philadelphia, PA (CA-18281); Jefferson Medical College, Philadelphia, PA (CA-14215); Mayo Clinic, Rochester, MN (CA-13650); University of Minnesota, Minneapolis (CA-20365); New York University Medical Center, New York, NY (CA-16395); University of Ottawa, Ontario; Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Pittsburgh, PA (CA-18653); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); University of Pretoria, South Africa (CA-21692); Swiss Group, Bern; SUNY–Stony Brook (Veterans Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Wisconsin Clinical Cancer Center, Madison (CA 21076); and Yale School of Medicine, New Haven, CT (CA-15485).

[3]We thank Ms. Joyce Greer for help in data management.

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## RESULTS

### Therapeutic Responses

No complete responses occurred in these 40 patients. Partial responses were noted in two patients and consisted of regression of pulmonary or abdominal metastasis (duration: 1 and 6 months, respectively). One of these patients had not received any prior therapy; the other one had received pelvic irradiation and combination chemotherapy with cyclophosphamide, doxorubicin, and cisplatin. Six patients had stable disease (duration: 2–5 months). All other patients had disease progression.

### Toxicity

Leukopenia ($\leq$ 2000 cells/mm$^3$), the most common dose-limiting toxic effect, was seen in five patients, one of whom died. Thrombocytopenia was less frequent. However, neither toxic effect seemed to be cumulative. Leukopenia nadir occurred within 1 week. Nausea and/or vomiting were generally mild to moderate and occurred in 17% of the patients treated. One patient developed transient hypotension related to rapid infusion. No patient experienced hepatic, renal, or cardiac toxic effects.

## DISCUSSION

No complete responses and only two partial responses occurred in 40 VM-26-treated patients. The treatment group included patients with good performance status, and 15 or 40 (37%) patients had received no prior chemotherapy. Because no significant tumor regression was achieved, it can be concluded that VM-26 in the dose used in this study is likely to have a response rate of $< 15\%$ ($P = 0.05$) (5). It appears to be of little therapeutic benefit in this dose schedule as a single agent in previously treated patients with metastatic transitional cell carcinoma of the urinary tract.

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# High-Dose Medroxyprogesterone Acetate (MPA) in Advanced Chemotherapy-Resistant Ovarian Carcinoma: A Phase II Study[1,2]

Clinical Trials Summaries

**Kristian Aabo,* Anders Gersel Pedersen, Inge Hald, and Per Dombernowsky[3]**

To further elucidate the value of high-dose progesterone in advanced chemotherapy-resistant ovarian carcinoma, 30 patients with progressive [International Federation of Gynecology and Obstetrics (FIGO) stage III and IV], histologically proven adenocarcinoma of the ovary were treated orally with medroxyprogesterone acetate (MPA) at a daily dose of 500 mg/m$^2$ in three divided doses. The patients had to be treated for a minimum of 4 weeks to be evaluable for response.

Response was evaluated by repeated physical and pelvic examinations and by chest X-rays. Abdominal computerized tomographic scans and ultrasound examinations were not used routinely but were done when clinically indicated. Pleural effusions, ascites, and osseous and CNS metastases were not acceptable as evaluable disease. Response was defined according to the World Health Organization criteria (1).

Twenty-seven evaluable patients had easily palpable pelvic and/or abdominal tumors. Patient characteristics are shown in table 1. The median duration of MPA treatment was 2 months (range, 1–6). Only one partial remission of 5 months' duration was achieved in a patient with stage IV cystadenocarcinoma. This patient had a > 50% reduction of metastatic neck lymph nodes and of an epigastric mass. Thus, the overall response rate was 3.7% (95% confidence limits, 0–18%). No toxic effects were observed.

The few reports on the use of progesterones in ovarian cancer have shown great variation in response. Jolles (2) reported four clinical responses among 10 patients (40%) with ovarian carcinomas treated with hydroxyprogesterone caproate, while Ward (3) found 15 of 23 patients (65%) responding to the same hormone. However, using MPA, Malkasian et al (4) found only one partial objective tumor regression among 19 patients (5.3%) treated with moderate doses.

The results in the present study are similar to those reported by Malkasian et al using the same progesterone derivative. Due to differences in compound, method of administration (oral vs im depot injections), and dose, the response rates cannot be directly compared.

MPA is readily absorbed from the gastrointestinal tract, and using the peroral high-dose sched-

TABLE 1.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Age (yrs) | |
| Median: 60 | |
| Range: 39–72 | |
| Time from diagnosis to start in study (mos) | |
| Median: 13 | |
| Range: 6–85 | |
| FIGO stage | |
| III | 22 |
| IV | 5 |
| Histology | |
| Cystadenocarcinoma | 24 |
| Endometrioid carcinoma | 2 |
| Solid adenocarcinoma | 1 |
| Previous chemotherapy* | |
| CAF | 14 |
| C | 2 |
| B | 11 |
| Overall response to chemotherapy | 16 (59%) |
| Previous radiotherapy | 3 |
| Response to MPA | 1 (3.7%) |
| MPA toxicity | 0 |

*C = cyclophosphamide; A = doxorubicin; F = 5-FU; and B = dihydroxybusulfan.

[1]Received July 2, 1981; revised Aug 26, 1981; accepted Sept 18, 1981.

[2]Medroxyprogesterone acetate (6-α-methyl-17-α hydroxyprogesterone acetate; Farlutal) was supplied as 100- and 200-mg tablets by Farmitalia-Carlo Erba, SPA, Milan, Italy.

[3]Departments of Chemotherapy (*K. Aabo* and *A. G. Pedersen*) and Gynecologic Oncology (*I. Hald*), Finsen Institute, and Medical Department C, Bispebjerg Hospital (*P. Dombernowsky*), Copenhagen, Denmark.

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ule, high serum concentrations are reached within few days (5). Therefore, the method of administration and dose requirements should secure a therapeutic level in the present study. The differences in the response rates, however, may be explained by the differences in defining response. Jolles and Ward did not use the WHO criteria as used in the present study and the study of Malkasian et al. Reviewing the 10 cases of Jolles according to the WHO criteria there may be one partial response and similarly only two to three partial responses of 2–3 months' duration among the 23 patients reported by Ward. These figures are within the 95% confidence limits found in the present study. It is therefore reasonable to conclude that responses of short duration may be obtained only in a few patients with advanced ovarian adenocarcinoma previously treated with chemotherapy.

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## Unusual Reaction to VP-16-213 and Avoidance by Prolonged Infusion

VP-16-213 (Etoposide) is a semisynthetic podophyllotoxin with antitumor activity against germ cell tumors, oat cell carcinoma of the lung, and lymphomas. The major limiting toxic effect is myelosuppression. There have been a few unusual, serious reactions associated with VP-16-213 (1–4). This case report documents another in a series of rare but potentially severe reactions to VP-16-213 administration. A new schedule of administration of VP-16-213 was instituted so that the drug could be continued.

A 16-year-old white female with a malignant teratoma of the ovary was begun on VP-16-213, doxorubicin, and cisplatin. Doxorubicin was given at a dose of 40 mg/m$^2$ on Day 1, cisplatin at 20 mg/m$^2$ on Days 1–5, and VP-16-213 at 100 mg/m$^2$ on Days 1–5 as a 1-hour infusion. During the second half of the Day-1 infusion of VP-16-213, the patient developed watery eyes, sneezing, and coryza, with no respiratory symptoms or findings. This reaction cleared as soon as drug administration was finished. VP-16-213 and cisplatin were given on Days 2–5 without further allergic symptoms. Twenty-eight days later, a second course of treatment was begun, with an identical reaction. A third course of the three drugs was administered with no reaction. On Day 1 of the fourth course, the patient reported pain in the throat and shortness of breath 30 minutes after the infusion was begun. The infusion was slowed, the symptoms subsided, and the VP-16-213 was completed and followed by a dose of cisplatin without problems. On Day 2, VP-16-213 was administered at a slower rate. After 1 hour of infusion, the patient reported severe pain in the throat and was unable to breathe. She became agitated and hoarse. Vital signs were stable during this episode, including rate and depth of respiration. No rales or wheezes were noted. The VP-16-213 was stopped and the symptoms gradually resolved. An ECG the following day was normal. Since the patient was in a complete remission, all chemotherapy was stopped. Four months later, because of progressive disease, VP-16-213 was reinstituted as a single agent. A dose of 25 mg/m$^2$ of VP-16-213 (50 mg diluted in 500 ml of normal saline) was given as a 6-hour infusion with no symptoms. The following day, 50 mg/m$^2$ (100 mg diluted in 500 ml of normal saline) was given over 6 hours with no symptoms. For the next 3 days, a dose of 100 mg/m$^2$ (200 mg diluted in 500 ml of normal saline, which is the manufacturer's current recommended dilution [B. F. Issel, personal communication] because it has been shown to be stable over a 6-hour period at that concentration) was given daily over 6 hours with no symptoms. Twenty-eight days later, the three-drug combination was again started with VP-16-213 at a dose of 100 mg/m$^2$ (200 mg diluted in 500 ml of normal saline) as a 6-hour prolonged infusion on Day 1, with no symptoms during its administration. On Days 2–4, VP-16-213 was given as a 4-hour infusion. Two further courses of the three-drug combination, with VP-16-213 as a 4-hour infusion, were similarly given with no side effects. No alteration in hematologic, gastrointestinal, or other toxicity was noted when VP-16-213 was given as a 4-hour infusion as opposed to the 1-hour administration. The patient had a partial response to this series of combination chemotherapy.

VP-16-213 has definite activity in a variety of tumors. However, it does have occasional serious side effects and frequent monitoring of pulse and blood pressure is indicated. Prolonging the administration time to 4–6 hours allowed us to continue the medication without further occurrence of any side effects. This form of administration may be helpful in other cases when the drug is indicated but when reactions have occurred.

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June 16, 1981

## Coronary Artery Disease in the Presence of Bleomycin Therapy

A 31-year-old male was seen in March of 1975 with a diagnosis of metastatic embryonal cell carcinoma of the left testicle.

The patient was treated with vinblastine (Velban) and a total dose of 225 mg of bleomycin. This treatment was temporarily discontinued because of intense diarrhea. The previously demonstrated metastatic disease disappeared after treatment.

Following completion of the 1-year vinblastine and bleomycin treatment, the patient was maintained on vincristine, cyclophosphamide, and methotrexate. He was treated for 1 additional year with these agents, totaling 24 months of treatment.

Six months after completion of chemotherapy (2½ years after the start and 1½ years after the completion of vinblastine and bleomycin therapy), the patient reported severe substernal chest pain unassociated with diaphoresis, cough, or paroxysmal nocturnal dyspnea (1). An ECG stress test was performed and was abnormal. Serum cholesterol was 201 mg/100 ml and triglyceride level was 84 mg/100 ml. Blood sugar had been normal. The patient underwent a coronary angiogram, and high-grade stenosis of the left anterior descending coronary artery was demonstrated (2). A saphenous vein graft to the left anterior descending artery was performed.

The patient has remained asymptomatic with regard to his coronary artery disease and has demonstrated no further evidence of neoplastic disease.

This patient is younger than those reported earlier (3), is a nonsmoker, and has no family history to suggest coronary artery disease.

Based on the foregoing, coronary artery disease secondary to the bleomycin and vinblastine combination must be suspected.

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July 6, 1981


## Severe Toxicity and Death Associated With Zinostatin Administration[1]

Zinostatin is a protein antibiotic that was first isolated and purified from *Streptomyces carzinostaticus* var. F47 by Ishida et al (1) in 1965. Initial clinical studies, performed principally in Japan, demonstrated activity against acute leukemia, gastric and pancreatic carcinomas, malignant melanoma, and breast cancer (2,3).

In July of 1977, the Cancer and Leukemia Group B (CALGB) initiated a phase II trial of zinostatin in patients with carcinoma of the bladder, kidney, pancreas, stomach, uterus, ovary, and liver and in those with acute leukemia and malignant melanoma. Zinostatin was given as a 5-day course every 35 days for patients with solid tumors. Previously untreated patients were given a dose of 2.25 mg/m²/day for 5 days. Patients who had received prior radiotherapy or chemotherapy were given a dose of 1.5 mg/m²/day for 5 days. Each dose was given as a 30-minute infusion. Patients with leukemia were given a dose of 2.5 mg/m²/day for 5 days, repeated at 7–10-day intervals.

A total of 183 patients were entered in the study. Allergic toxicity was graded according to CALGB criteria as either grade 0 (no toxicity), grade 1 (transient rash, fever $< 38°C$, no treatment required), grade 2 (urticaria, fever $> 38°C$, treatment required), grade 3 (wheezing and/or upper airway obstruction), or grade 4 (anaphylaxis).

Of the 183 patients, 17 (9%) were considered to have had mild to moderate hypersensitivity reactions (grade 1, 2, or 3), which included rash, fever, urticaria, or mild wheezing. Nine patients were considered to have had a grade 4 reaction with severe respiratory distress and/or hypotension.

The characteristics of these nine patients are summarized in table 1. Overall, the major manifestation of toxicity in five of these nine patients was hypotension. In four patients, the major symptom was severe respiratory distress only. Hypotension always occurred on the first day of treatment of the second or third 5-day course. Dyspnea without hypotension occurred on the first day of the first course and on the second day of the second course in three of the four patients.

Of the 17 instances of major toxicity, one resulted in death. This death occurred in a 65-year-old female who was given zinostatin for metastatic renal cell


[1]Supported in part by Public Health Service grants CA-16450, CA-03927, CA-25119, CA-21060, CA-12046, CA-04457, CA-08025, CA-07968, CA-04326, and CA-26806 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

TABLE 1.—Characteristics of onset and symptoms in patients with severe grade 4 reactions

| Patient No. | Onset of symptoms: Course No. | Onset of symptoms: Day | Respiratory distress | Hypotension | Outcome |
|---|---|---|---|---|---|
| 1 | 1 | 1 | + | + | Dead |
| 2 | 3 | 1 | + | 0 | Alive |
| 3 | 3 | 1 | + | + | Alive |
| 4 | 1 | 1 | + | 0 | Alive |
| 5 | 3 | 1 | 0 | + | Alive |
| 6 | 2 | 2 | + | 0 | Alive |
| 7 | 2 | 1 | + | + | Alive |
| 8 | 1 | 1 | + | 0 | Alive |
| 9 | 3 | 1 | 0 | + | Alive |

carcinoma. Thirty days after her first course, the patient began a second course of therapy. Within 5 minutes of beginning the zinostatin infusion, the patient became agitated, vomited, and reported shortness of breath. Within 30 seconds of her first symptoms, she was unconscious, cyanotic, and pulseless. Cardiopulmonary resuscitation was begun. An initial cardiac asystole converted to sinus tachycardia. However, despite continued efforts, the patient became increasingly hypoxic and died after 40 minutes. An autopsy was done; death was attributed to acute pulmonary edema.

Since zinostatin is a polypeptide antibiotic, there has been just concern regarding possible sensitization and subsequent allergic reactions. In the current study, five patients developed hypotension that always occurred with the first daily dose of the second or third course of the drug. Thus, hypotension in this series always, and only, occurred in patients who had prior exposure to the drug and thus appears to be a result of prior sensitization. In the literature, hypotension following zinostatin was observed on the first day of the third course (4) and on the first day of the second course (5) in two patients.

Zinostatin, as indicated by these data, may result in hypersensitivity reactions, which, especially after the first course, may be severe and life-threatening.

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July 20, 1981



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# ANNOUNCEMENTS

## World Congress of Gastroenterology, Digestive Endoscopy, and Coloproctology

***Stockholm, Sweden, June 13–19, 1982***

Information on this international meeting can be obtained from the Stockholm Convention Bureau, Jakobs Torg 3, S-111 52, Stockholm, Sweden.

## Third Annual Meeting of the American College of Clinical Pharmacy

***Kansas City, Missouri, June 25–26, 1982***

This meeting on pharmacotherapeutics and pharmacokinetics will be held at the Crown Center. The deadline for submitting abstracts is March 1, 1982. Abstract forms are available from: Darwin Zaske, St. Paul-Ramsey Medical Center, 640 Jackson St, St. Paul, MN 55101 (telephone: 612–221-3456).

## Fourteenth Meeting of the International Society for Pediatric Oncology (SIOP)

***Berne, Switzerland, September 21–25, 1982***

The main theme of this meeting will be new modes of therapy and supportive care. Specific topics will include immune modulation, clinical applications of monoclonal antibodies, bone marrow transplantation, infection and nutrition, and new modes in surgery and radio-oncology. There will be reports on current trials as well as a pathologists meeting. The meeting, to be conducted in English, will be held at the Department of Pediatrics, Inselspital, 3010 Berne, Switzerland.

*For further information contact*: Hans P. Wagner, MD, c/o SPOG, Institute for Clinical and Experimental Cancer Research, Tiefenauspital, 3004 Berne, Switzerland.

## Third EORTC Breast Cancer Working Conference

***Amsterdam, The Netherlands, April 27–29, 1983***

The focus of this meeting, sponsored by the Breast Cancer Cooperative Group of the EORTC, will be clinical problems related to breast cancer. Review lecturers and parallel workshops are planned as well as ample time for discussion. The conference language will be English. Participation is open to physicians in all fields of medicine interested in the biology and treatment of breast cancer.

*For further information contact*: Conference Secretariat, c/o Congress Bureau, Oudezijds Achterburgwal 199, 1012 DK Amsterdam, The Netherlands.

## Second International Drug Conference

***Edinburgh, Scotland, September 21–23, 1983***

The theme of this meeting will be rate control in drug therapy. Further details are available from: The Secretariat, Second International Conference on Drug Absorption, Centre for Industrial Consultancy and Liaison, University of Edinburgh, 16 George Square, Edinburgh EH8 9LD.

## Position Vacancy: Associate Director, Cancer Therapy Evaluation Program

The position of *Associate Director of the Cancer Therapy Evaluation Program* is now vacant. This position is located within the Division of Cancer Treatment, National Cancer Institute (NCI), National Institutes of Health, Public Health Service, Department of Health and Human Services.

This is a civil service position in the Senior Executive Service (SES), with a salary range from $54,755 to $61,300 (currently limited to $58,500 per annum). Candidates may be eligible for Physicians Comparability Pay up to $10,000 per year. (Alternatively, candidates may be eligible for appointment in the Commissioned Corps of the US Public Health Service.) The individual selected, if not presently in SES, must serve a 1-year probationary period.

The NCI develops the overall national strategy against cancer, coordinates federal government and nonfederal government cancer programs, and supports international programs of cancer research.

The Division of Cancer Treatment is responsible for directing the NCI's major research effort related to the treatment of cancer. The Cancer Therapy Evaluation Program monitors and sponsors clinical trials for cancer therapies. This extramural program supports the investigation of therapeutic modalities through the use of grants, contracts, and cooperative agreements. In fiscal year 1981 the program had a budget of approximately $128,000,000 and a staff of 50 persons.

The mandatory professional/technical qualifications for the position require that candidates:

1. meet the minimal educational requirements for a Medical Officer, GS-602 as defined by the US Office of Personnel and Management Handbook X-118 (Qualification Standards for Positions Under the General Schedule), which is available in federal personnel offices;
2. have clinical and biomedical research experience and knowledge of research programs related to the treatment of cancer. Clinical experience must include the management of all treatment modalities: surgery, radiotherapy, medical oncology, and biologic response modifiers. Candidates must have participated in clinical trials using at least one of the mentioned modalities and must have managed trials of a multimodal nature; and
3. be licensed and qualified to provide care to patients in NCI research clinical trials.

The managerial/executive qualifications are experience as director of a clinical research program of substantial size, including the specialties surgery, radiotherapy, and medical oncology, and demonstration of competence to assume leadership responsibilities in:

1. assuring that national and agency goals and priorities are considered in carrying out the functions and responsibilities of the organization;
2. establishing and maintaining relationships with key individuals and groups outside the organization;
3. directing and guiding programs and projects, including long-term and short-term planning and establishing objectives and priorities;
4. establishing procedures for the development and defense of the organization's budget, and allocating fiscal and personnel resources to support program implementation;
5. developing and implementing procedures for monitoring program performance, including periodic analysis and evaluation; and
6. developing processes and overseeing activities to implement EEO objectives and to assure the fair and equitable employment of qualified persons.

Candidates who meet the mandatory requirements will be evaluated by the search committee on the following factors: scientific reputation/peer recognition, training and education, honors and awards, and the degree to which they exceed the minimally qualified level of the mandatory professional/technical and managerial/executive qualifications.

Applications (personal qualifications statement SF-171) are to be sent to: Ms. Susan Corey, National Cancer Institute, Bldg 31, Rm 3A34, Bethesda, MD 20205. *Applications must be postmarked by March 1, 1982.* A current curriculum vitae and bibliography must accompany all applications. Applications of women and/or minority group members are especially solicited. The National Institutes of Health is an equal opportunity employer.

*Additional information may be obtained from*: Ms. Susan Corey, Personnel Management Specialist, NCI (301–496-6862).

## Cancer Control and the Primary Physician

***Summit, New Jersey, March 3, 1982***

This 1-day symposium, sponsored by Overlook Hospital, Columbia University Comprehensive Cancer Center, and the New Jersey Division of the American Cancer Society, will be held at Overlook Hospital, Summit, New Jersey, and is open to physicians, nurses, and other health care professionals. Topics to be covered include: cancer statistics in New Jersey and what they mean, screening, pain control, the role of nutrition, comprehensive care of the cancer patient, psychosocial support, hospice/home care, and rehabilitation. Symposium coordinators are Drs. William F. Minogue and Robert N. Taub. Guest faculty include Drs. Susan Mellett, Alfred I. Neugut, Steven Stellman, Arthur Taub, and Myron Winick.

The course has been approved for 6 CME credits by the Medical Society of New Jersey, American Academy of Family Practice, Academy of Medicine of New Jersey, and the New Jersey State Nurses Association (pending).

*For further information, contact*: Mrs. Cordis L. Griffith, Department of Medical Education, Overlook Hospital, Summit, NJ 07901 (201–522-2085).



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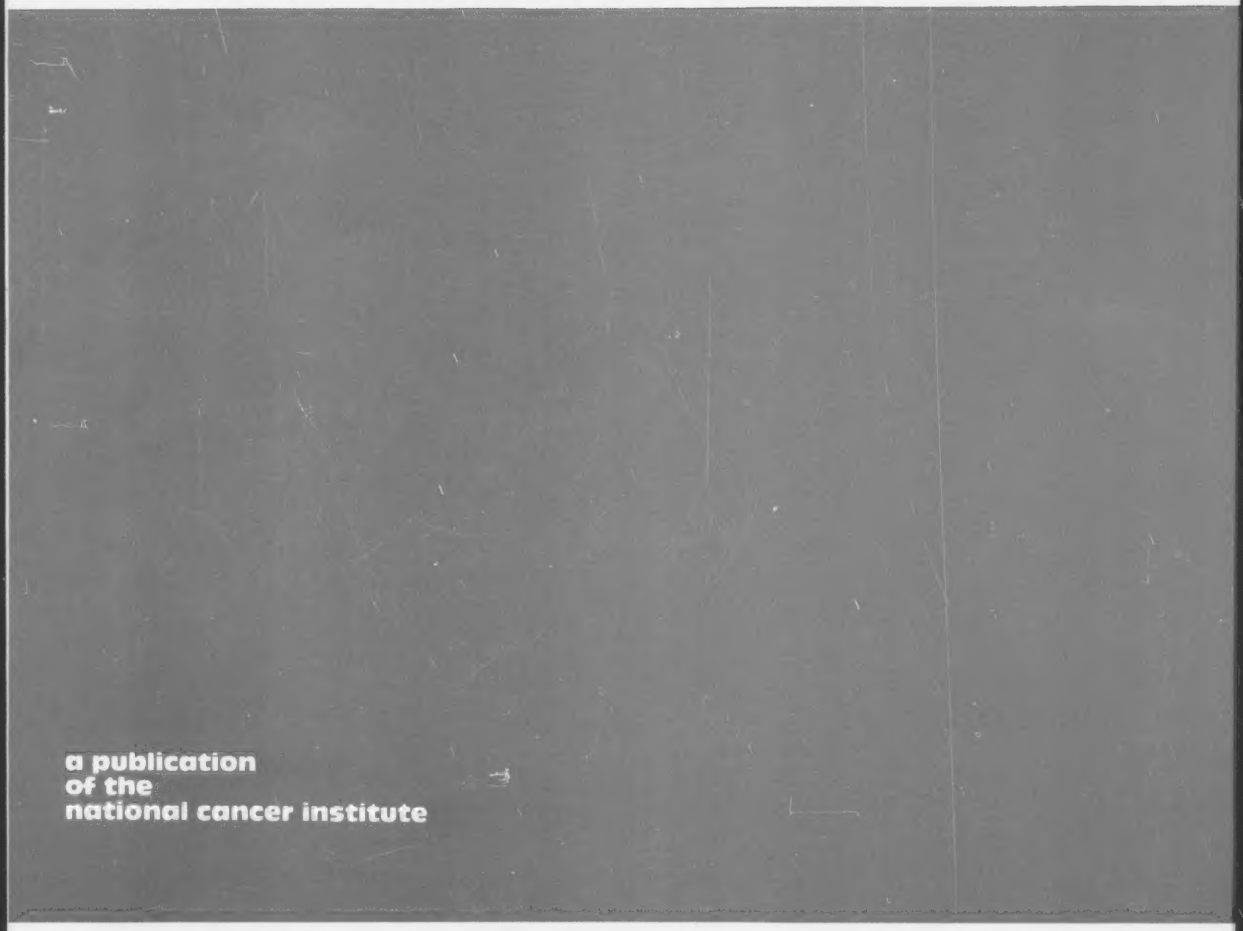

a publication
of the
national cancer institute

NIH Publication No. 82-22